Analyses, persistence and degradation of the synthetic pyrethroid insecticides permethrin and fenvalerate

by

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To my parents

Abstract

Studies on persistence and degradation of the synthetic pyrethroid insecticides, permethrin and fenvalerate, were carried out under natural environmental conditions of the Niagara Peninsula. Permethrin and fenvalerate were treated on apple foliage at rates of 0.21 kg(AI)/ha and 0.14 kg(AI)/ha, respectively. The initial cis- and trans-permethrin spray deposits were found to be 13.5 ppm and 19.2 ppm, respectively and 38.0 ppm was observed for the fenvalerate treated sample. Twenty-three days and 84 days after spray application, permethrin residues were 4.0 ppm and 2.7 ppm for the cis-isomer, whereas they were 7.9 ppm and 4.7 ppm for the trans-isomer, respectively. Residues of fenvalerate 23 days and 84 days after spray application were 13.4 ppm and 8.0 ppm, respectively. The values of observed half-life of cis-permethrin, trans-permethrin and fenvalerate were found to be 42 days, 46 days and 51 days, respectively. Studies were extended to quantitatively determine some of the major degradation compounds of permethrin and fenvalerate, which were expected to be produced as results of ester cleavage of the parent compounds. A permethrin treated sample, 84 days after initial spray application, showed 0.25 and 0.8 ppm of cis- and trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid (Cl_2CA (18)), respectively. These two acids were not found as free acids, but found as conjugated compounds. The other expected degradation compounds, 3-phenoxybenzyl alcohol (PBalc (19)),

3-phenoxybenzaldehyde (PBald (38)) and 2-(4-chlorophenyl)isovaleric acid (CPIA (31)) were not detected by the methods employed in this study. The results indicate that these degradation compounds were not present, or, if they were present, their concentrations were too low to detect by the methods used.

Acknowledgements

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Introduction

I. Brief history of chemical pesticides

The use of chemicals to control pests dated to the ancient Greeks, who used sulfur against insects and sodium chloride (sea salt) against weeds.[1] The naturally occurring pesticide isolated from chrysanthemum cinerariae-folium had long been recognized as an excellent insecticide because of its valuable properties such as acting rapidly against a wide range of insects, and leaving no harmful residues in the environment. Although it has a favorable insecticidal character, it is unsuitable for field use as an insecticide because of its instability to air and light.

In the late 1800's, farmers began using a variety of pesticides such as the naturally occurring compound, nicotine, which was used to control aphids.[2] The first synthetic organic pesticide, potassium dinitro-o-cresylate was used as an insecticide and as a herbicide.[1] Inorganic poisons such as hydrogen cyanide, lead arsenate, a variety of mercury compounds, and Paris green (copper acetoarsenate) which was used for the control of the Colorado potato beetle, were also introduced. These metal-containing compounds were not very effective as insecticides and were hazardous to humans because of their persistence and poisonous nature.

After World War II, new pesticides for insects, plant disease and weed controls were discovered in a remarkably rapid fashion. These pesticides were used not only for increasing the food supply, but also for control of malaria and a host of other tropical diseases.[3] Some of these

pesticides are highly stable under ordinary environmental conditions. For example, the first synthetic organic insecticides, DDT (dichlorodiphenyl-trichloro ethane) and dieldrin were discovered not only in the treated areas, but also at remote non-target regions. Moreover, DDT has a half-life of 15 years or longer in some soil types and a maximum of 55 days half-life on plant surfaces.[4] The repeated use of very persistent organochlorine compounds created insect resistance to these chemicals, widespread distribution throughout the environment, accumulation in animal fats and biological magnification in food chain organisms. These problems alerted the public to concern about the possible long-term effects of the large quantities of insecticides being used.[5] The development of insecticides with high insecticidal activity and low mammalian toxicity was continued, and by the early 1970's several synthetic products with favorable characteristics were discovered, some of which are synthetic pyrethroids.

II. Classification of pesticides

The pesticides can be classified according to the chemical nature and the mode of action against the pests. For example, insecticide, fungicide, herbicide, nematocide and acaricide are the pesticides having typical action against insects, fungi, weeds, nematodes and mites, respectively. Some of the common pesticides are listed in Figure 1.

In general, insecticides can be characterized into three major groups which are organochlorine, organophosphorus and carbamate, according to their similarities of chemical structure as shown in Figure 2.[2]

Figure 1. Pesticide structures and their typical actions

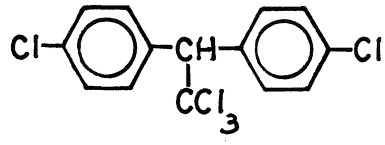
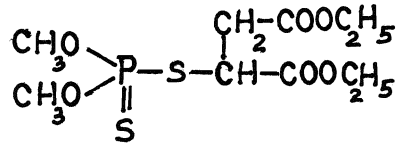
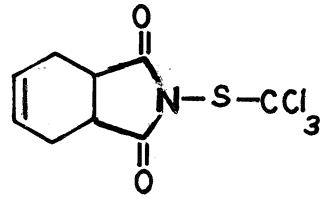
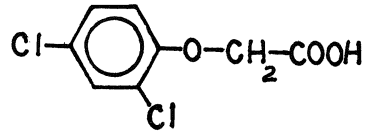
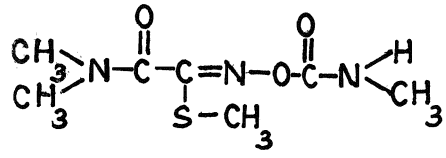
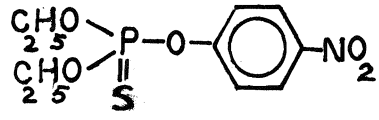
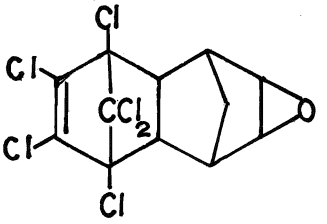
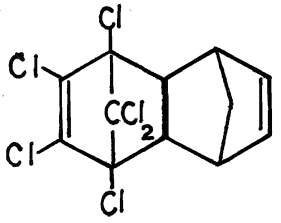
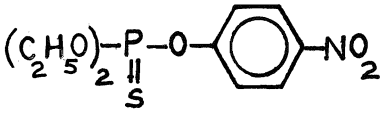
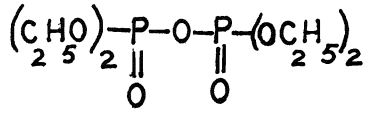
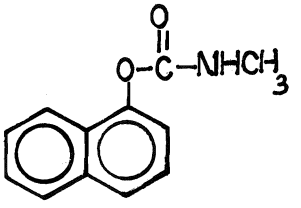
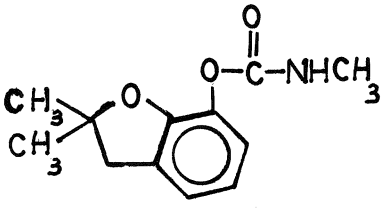
Compound	Structure	Typical action
DDT (dichlorodiphenyl-trichloroethane)		insecticide
malathion		insecticide
captan		fungicide
2,4-D (2,4-dichloro-phenoxyacetic acid)		herbicide
oxamyl		nematocide
parathion		acaricide/ insecticide

Figure 2. Classification of insecticides

Classification	Compound	Structure
organochlorine	dieldrin	
	aldrin	
organophosphorus	parathion	
	TEPP (tetraethyl pyrophosphate)	
carbamate	carbaryl	
	carbofuran	

Organochlorine insecticides are characterized by the presence of one or more chlorine atoms substituted for hydrogens on carbon. These chlorinated hydrocarbons do not occur in nature. Most of them have a similar property: persistence in the environment. They have very limited solubility in water, but are very soluble in fat, and can undergo biological magnification in the food chains. Although organochlorine compounds are known to be very stable, their toxicity to man is relatively low in comparison with organophosphorus and carbamate compounds, and there is no evidence revealed that anyone has died of chronic illness cause by these residual insecticides.[4] Despite the low mammalian toxicity of organochlorine compounds, the long-term effects of their persistence and accumulation were a cause for concern and had been a major factor when many countries decided to ban the use of some of the chlorinated hydrocarbons in the beginning of the 1970's.

The organophosphorus compounds are relatively non-persistent, but they are considered to be more toxic than the other classifications of pesticides. These molecules have the ability to act as nerve toxins by the following action.[1] A substance known as acetylcholine is responsible for the transmission of nerve impulses from one nerve cell to another. After serving its function, the acetylcholine must be destroyed by the enzyme, acetylcholinesterase, in order to enable the next nerve impulse to flow. Organophosphorus insecticides inhibit the ability of the enzyme to function. As a result, acetylcholine accumulates and activates a stream of undesired nerve impulses which can lead to death. However, though these compounds are more toxic, they are non-persistent under ordinary environmental

conditions, thus they are considered safer than organochlorine compounds from the environmental pollution viewpoint.[4]

The persistence of carbamate compounds is similar to that of organophosphorus compounds, and they are also more toxic than the chlorinated hydrocarbons. The pesticidal action of carbamate is analogous to that of organophosphorus compounds.

III. Brief history of pyrethroids

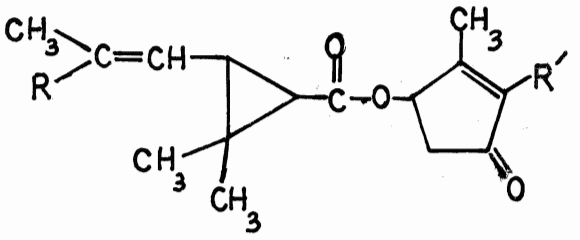
The valuable insecticidal properties of pyrethrums, the dried flowers of chrysanthemum cinerariaefolium, have been known since the nineteenth century.[2] The insecticidal principles of pyrethrums are called "pyrethrins", and long had been considered harmless to mammals and plants while very toxic to insects.[6] Pyrethrins were obtained by extracting the pyrethrums with kerosine, followed by dewaxing and concentrating the extract. Then the resulting petroleum solution was re-extracted again with nitromethane. The yield of pyrethrins obtained from this process was over 90%.[7-9] These high yields of pyrethins could be obtained only when the flowers were hand-picked in their full bloom. The quantity of active pyrethrins in pyrethrum varied from one country to another. For example, the Kenyan product had about 1.3% active ingredient whereas 0.9% was found in the Japanese product.[6]

Although the detailed chemical study of the insecticidal principles of pyrethrum flowers was reported by Fujitani in 1909,[10] the structures of active materials contained in pyrethrins were unknown. The extensive

investigations and structure determinations of Staudinger and Ruzicka[11] deduced that there were two different compounds, pyrethrin I and pyrethrin II, the esters of chrysanthemic and pyrethric acid, respectively. LaForge and Barthel[12] separated two more pyrethroids, in 1944, from pyrethrum extracts named as cinerin I and cinerin II. As a result of the progress of chromatography, Godin and co-workers,[13-15] isolated two additional pyrethroids in 1964, jasmolin I and jasmolin II, from pyrethrum extract, and elucidated their structures. Finally, a century of chemical investigation disclosed that the insecticidal properties of pyrethrum were due to six structurally-related esters,[16] and they are listed in Figure 3.

All these esters were cyclopropane carboxylic acid esters and $-COOCH_3$ groups were at the unsaturated side chain on C-3 were trans- to the carboxyl group in the natural esters. Pyrethrins had very effective knockdown action even in small dosages. The knockdown action occurs almost instantaneously but its effect does not last very long. In order to kill insects, a very high dosage of pyrethrins is required. However, pyrethrins show very low toxicity to mammals when applied dermally or orally.[6] These characteristics of pyrethrins made them valuable for household use as livestock sprays, and for stored grains, vegetables and fruits. However, they were highly unstable to light and deteriorated very rapidly, hence they were not applicable for agricultural use. The high cost of production of natural pyrethrins and their instability to light stimulated the development of synthetic pyrethroids to replace the natural insecticides.

Figure 3. Structures of pyrethrins and related compounds[2]



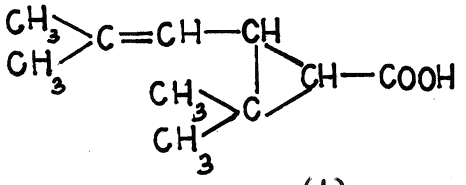
Natural pyrethroids	R =	R' =
pyrethrin I	$-CH_3$	$-CH_2CH{=}CHCH{=}CH_2$
pyrethrin II	$-COOCH_3$	$-CH_2CH{=}CHCH{=}CH_2$
cinerin I	$-CH_3$	$-CH_2CH{=}CHCH_3$
cinerin II	$-COOCH_3$	$-CH_2CH{=}CHCH_3$
jasmolin I	$-CH_3$	$-CH_2CH{=}CHCH_2CH_3$
jasmolin II	$-COOCH_3$	$-CH_2CH{=}CHCH_2CH_3$
Synthetic pyrethroid		
allethrin	$-CH_3$	$-CH_2CH{=}CH_2$

1. Development of synthetic pyrethroids

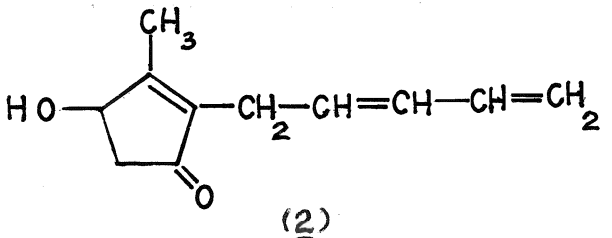
The field of synthetic pyrethroids with structures related to the natural pyrethrin developed very rapidly by modifying the framework of acid and alcohol moieties. The eminent pioneers of pyrethrum chemistry, Staudinger, Ruzicka and Yamamoto reported that the constituent alcohols and acids were active only when combined with one another and that an intact ester-linkage was important.[17]

In 1949, the first synthetic pyrethroid, allethrin (as shown in Fig. 3), was synthesized by LaForge and Barthel[18,19] by shortening the dienic unsaturation in the alcohol side chain of pyrethrins. In the study they reported that the insecticidal activity of allethrin was as effective as pyrethrin and their mammalian toxicities were also comparable. For example, the acute oral toxicity LD_{50} for allethrin is 300-650 mg/kg to mice, whereas it is 330-720 mg/kg for pyrethrins. Since the photostability of allethrin was improved only marginally, it has still not reached the level of field use.

Several synthetic and biological studies on the analogs of the natural esters had been carried out simultaneously. Staudinger and Ruzicka[20] synthesized acids structurally similar to chrysanthemic acid (1) and esterified them with pyrethrolone (2) and also synthesized alcohols analogous to pyrethrolone and esterified them with chrysanthemic acid. Only a few of the esters showed toxicity comparable to the natural pyrethrins. In searching for new biologically active esters, the pyrethrolone moiety was replaced by various alcohols having no relation



(1)



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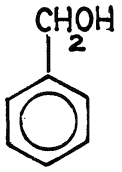

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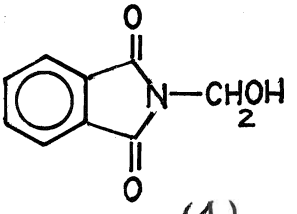

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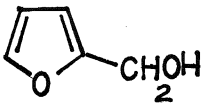

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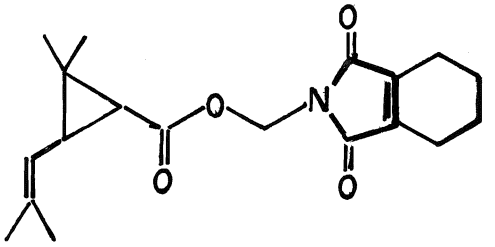
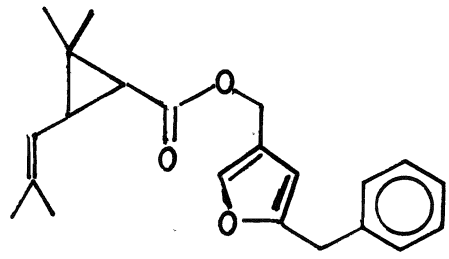


(6)

to pyrethrolone. Benzyl alcohol (3), phthalimidocarbinol (4) and furylcarbinol (5) were found to have good insecticidal activities when esterified with chrysanthemic acid. None of the esters of modified acids were found to be as toxic as pyrethrins until the discovery of 2,2,3,3-tetramethylcyclopropane carboxylic acid by Matsui and Kitahara,[21] who synthesized various esters of this acid and found them nearly equal to chrysanthemates in their toxicity to insects.[22]

By 1968, tetramethrin (6), a good knockdown agent, and resmethrin (7), the first synthetic compound with greater insecticidal activity and lower mammalian toxicity than the natural esters, had been discovered. However, these compounds did not greatly extend the range of application of pyrethroids because they were unstable, like the natural compounds. Investigation of structure-activity relationship continued, and by 1973 several new compounds had been developed which were more photostable yet retained many of the favorable characteristics of the natural esters and earlier synthetic compounds. Among the new synthetic pyrethroids, permethrin (8), fenvalerate (9) and cypermethrin (10) have received special attention because of their persistence, which is long enough to give adequate control of insects in agricultural use, yet it is not long enough to create environmental problems. The comparison of the activities of pyrethroid with some other insecticides[17] is shown in Table 1.



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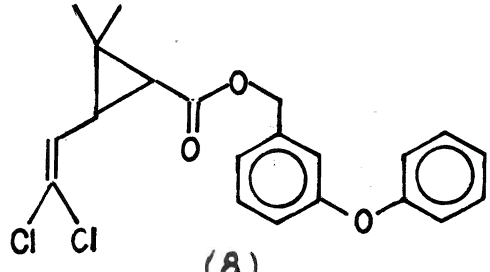

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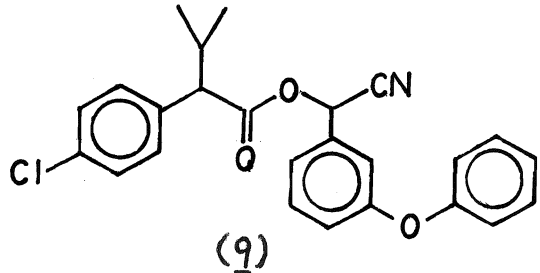

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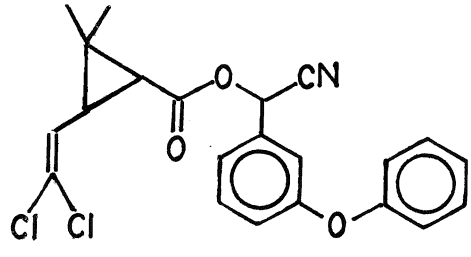


(10)

Table 1. Toxicities of classes of insecticides to insects and mammals[17]

Class	Rat (mg/kg)	Insects (mg/kg)
carbamate	45	2.8
organophosphorus	67	2.0
organochlorine	230	2.6
pyrethroid	2000	0.45

Permethrin (8) was the first synthetic compound which has adequate stability for field use.[23] It has several favorable characteristics, i.e., excellent insecticidal activity, low mammalian toxicity, moderate persistence and left no harmful residues in the environment. In this compound, the methyl groups in the isobutenyl side chain were replaced by chlorine atoms which raised the insecticidal activity of cyclopropane carboxylate. Similarly, the alcohol component was substituted with a 3-phenoxybenzyl group, which was more photostable than pyrethrolone or other previous alcohols.

A further important stage in the evolution of pyrethroids for use in agriculture was the discovery of the active ester, fenvalerate (9), which had the composition of α-cyano-3-phenoxybenzyl alcohol with a non-cyclopropane acid such as α-isopropyl-4-chlorophenylacetic acid. This product was developed by Ohno and co-workers[24,25] and it also has many valuable characteristics like permethrin's.

The recent development of outstanding synthetic pyrethroids, permethrin and fenvalerate, led to their important potential applications against agriculture and forest insect pests, and therefore at present

they are the subjects of intense interest throughout the world.

2. Mode of action of pyrethroids

Pyrethroids are considered as contact poisons because they are principally active by contact and are later transmitted readily to all kinds of tissues by penetrating through the cuticle of insects into the hemolymph, then rapidly spreading throughout the body of the insects and poisoning them.[26]

Although the prominent biological characteristic of pyrethroids is the very rapid knockdown of insects even in small dosages, insects usually recover after a short period of time. This recovery is due to rapid enzymatic detoxication in the insects.[2] Despite a great deal of investigation, however, very little is known about how pyrethroids produce their effect. An obvious, but incomplete, conclusion is that the knockdown may be caused by some sort of neurotoxic action.[27-31]

IV. Physical and chemical properties of permethrin

Permethrin (8), 3-phenoxybenzyl(±)cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate is sold under several code numbers by various companies that prepare it, e.g., NRDC 143, WL 43479, FMC 33297, and it is also known as Ambush (Chapman). It is one of the newest synthetic pyrethroids, and was developed by Elliott and his associates[23] at Rothamsted, England in 1972.[17] Because of its outstanding characteristics, it has great potential as an agricultural insecticide, low mammalian toxicity and adequate stability in air and light,[23] it was registered in Canada for

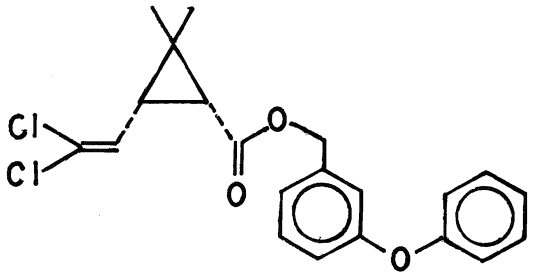
pre-season spray for apples and pears in 1978.

As the geometrical restriction of the cyclopropane ring at two of the chiral centres, C-1 and C-3, permethrin has four stereoisomers, two cis and two trans isomers as shown in (11)-(14). These isomer ratios affect significantly the chemical and biological properties.[33] In this study, permethrin contained the cis:trans isomers in an approximate 40:60 ratio. In general, high insecticidal activity is associated with the (R)- configurations of the chiral cyclopropyl carbon, C-1 adjacent to the carboxyl group. Whereas the enantiomers having the opposite configuration (S)-isomers are only slightly toxic.[17,34] One study[33] reported that cis-permethrin is more insecticidally potent than the trans-isomer and the study also showed that there are significant differences in rates of photolysis and hydrolysis of these isomers.

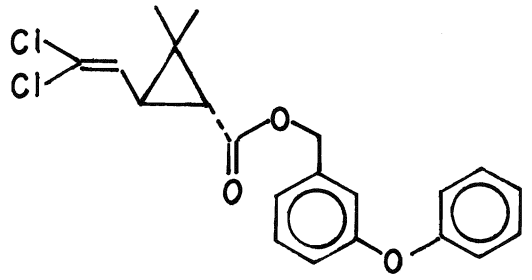
Permethrin is a yellowish brown oily liquid and its density at ambient temperature is in the range of 1.09-1.26 g/cm^3. The solubility of permethrin in various solvents at 0°C and 20°C is summarized in Table 2.

Table 2. Solubility (g/L) of permethrin[35]

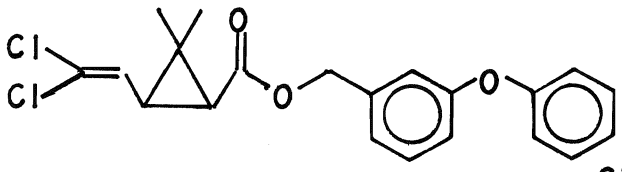
Solvent	0°C	20°C
hexane	>450	>450
chloroform	>450	>450
acetone	>450	>450
dimethylformamide	>450	>450
ethanol	222	>450
methanol	213	310
water	--	<0.001



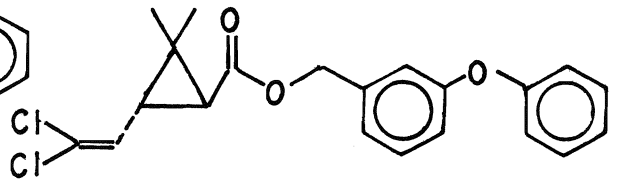
(11) 1R (CIS)



(12) 1R (TRANS)



(13) 1S (CIS)



(14) 1S (TRANS)

From the table, it may be noted that permethrin is very soluble in nonpolar organic solvents, decreases its solubility in polar organic solvents and has very low solubility in water.

The stability of permethrin under acidic, neutral and alkaline conditions was measured by its percentage recovery,[35] as shown in Table 3. For the stability study, a known quantity of permethrin was dissolved in water/acetone solution together with universal buffer solution at the reported pH's. Each solution was kept at 75°C for 100 hours, then cooled to ambient temperature and extracted with hexane. The hexane extract was dried over anhydrous sodium sulphate and introduced to a gas liquid chromatograph for analysis.

Table 3. Stability of permethrin at various pH's

pH	% recovery
2	92
4	96
6	94
8	88
10	2
12	0

The above data indicate that permethrin has a hydrolytic stability and is reacted at pH's 10 or greater. The study[35] of thermal stability reported that permethrin decomposed at temperatures above 220°C.

Permethrin showed a wide spectrum of activity and is principally active by contact. Unlike most of the organophosphorus insecticides, permethrin does not have systemic property, the ability to penetrate the

tissues of the plant and translocate to different parts remote from the site of application. There are no cases of phytotoxicity that have been reported due to the use of permethrin.

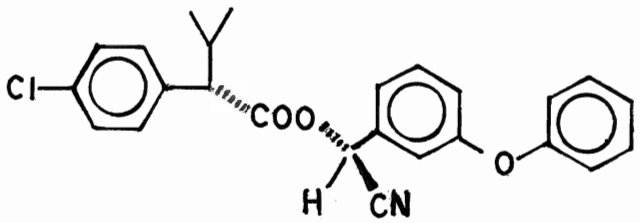
Compared with natural pyrethrins, permethrin was less hazardous in mammals.[36] Elliott et al.[37] and Gaughan et al.[38] studied the metabolism of permethrin in rat and cow and found that the parent compound hydrolysed quickly and converted enzymatically to more polar compounds and was excreted almost completely from the body of tested mammals within a short period of time. In a separate study, Elliott et al.[39] reported acute oral LD_{50} to rat as being 2000 mg/kg. These features make this compound a potentially safe insecticide, but the toxicological studies[40,41] reported that permethrin is more toxic to fish than the natural pyrethrins.

V. Physical and chemical properties of fenvalerate

Fenvalerate (9), alpha-cyano-3-phenoxybenzyl-2-(4-chlorophenyl)-isovalerate (also known as Sumicidin (Sumitomo), S5601 (Sumitomo), WL43775 (Shell), Belmark (Shell, Canada) and Pydrin (Shell, US), belongs to a new group of synthetic pyrethroid esters and it has a structure in which the cyclopropyl ring of permethrin is replaced with an isobutyl group to which is attached a 4-chlorophenyl group, and retaining a cyano group substitution on the benzyl carbon of the 3-phenoxybenzyl group. Although fenvalerate does not contain a cyclopropane ring in its chemical structure, its mode of action against insects and the toxic effects in mammals are very similar to other synthetic pyrethroids.[25,42,43] Fenvalerate was

registered in Canada in 1980.

Fenvalerate has two chiral centres, at C-2 of the acid moiety and at the α-C of the alcohol moiety, and therefore, four isomers designated RR, SS, RS and SR. The isomers with the acid moiety in the S-configuration, RS and SS, are insecticidally active,[44] and of these, the SS isomer (15) is the most active form of fenvalerate.[45,46]



(15) SS isomer; [2S,αS]

Fenvalerate is a yellow oil with the density of 1.17-1.26 g/cm^3 at 23°C[47]. Like permethrin, fenvalerate has a very low solubility in water, but is highly soluble in many organic solvents, as shown in Table 4.

Table 4. Solubility (g/L) of fenvalerate[48]

Solvent	0°C	20°C
hexane	49	77
chloroform	>450	>450
acetone	>450	>450
dimethylformamide	>450	>450
ethanol	>450	>450
methanol	225	>450
water	--	<0.001

The study of hydrolytic stability indicated that fenvalerate is unstable in alkaline media; results are similar to those of permethrin, as shown in Table 5.

Table 5. Hydrolytic stability of fenvalerate[47]

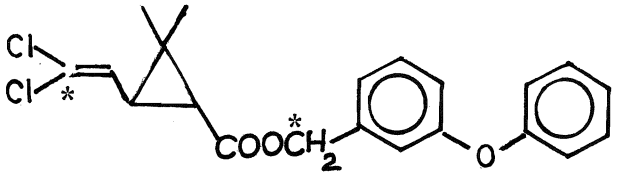
pH	% recovery
2	100
4	100
6	93
8	29
10	0
12	0

Fenvalerate is relatively involatile with vapour pressure 2.1 x 10^{-6} mm Hg at 70°C[47] and is thermally stable, decomposing at temperatures above 220°C. Its photochemical stability is moderately high, about three times higher than that of permethrin under simulated sunlight. This higher photostability could probably be due to the presence of aromatic rings in both acid and alcohol components.

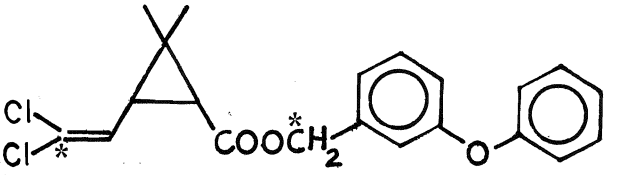
Similar to permethrin, fenvalerate is a highly active broad spectrum insecticide. Particularly, it is effective as a contact and stomach poison against lepidoptera, heritera and diptera larvae.[49] The short-term dietary studies in dogs and long-term studies in rats and mice showed that no carcinogenic effect was observed,[34] and it was rapidly absorbed, distributed to a variety of tissues and organs, metabolized and excreted from the body. The half-life for excretion was 0.5-0.6 day. The acute oral toxicity of fenvalerate to rats was found to be LD_{50} = 451 mg/kg. A temporary acceptable daily intake for man was set at 0.06 mg/kg. Like permethrin, fenvalerate is also very toxic to fish and bees.[47]

VI. Fate of permethrin in soil

Kaneko and co-workers[50] studied the degradation and movement of (+)-trans and (+)-cis isomers of permethrin in two Japanese soils under laboratory conditions. In the study, (+)-trans and (+)-cis isomers of permethrin labelled with ^{14}C in the alcohol and acid moiety, as shown in (16) and (17), were applied to two types of soils at the rate of 1.0 ppm and held at 25°C in the dark. They found that both isomers were rapidly decomposed with half-lives of 6 to 12 days. The degradation pathways for permethrin isomers in the soil are shown in Figure 4. Both isomers underwent mainly hydroxylation at the gem-dimethyl group of the acid moiety, at the phenoxy group of the ester and the alcohol moiety, cleavage of the ester linkage and oxidation of benzyl alcohol to benzoic acid.



(16) (+)-trans-permethrin

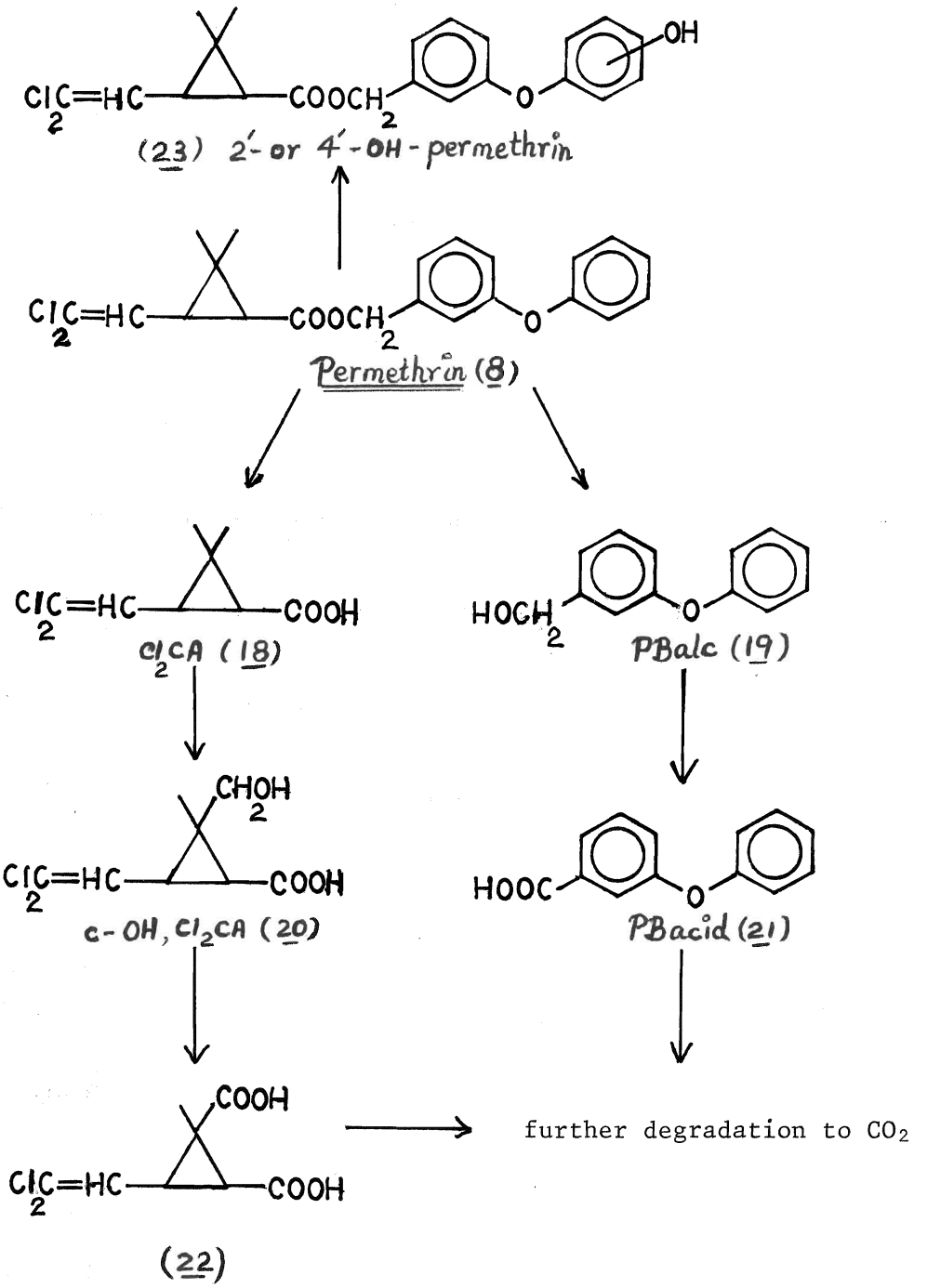


(17) (+)-cis-permethrin

* denotes the labelling positions

The sum of these identified degradation products was larger with cis-permethrin than with trans-permethrin, although the degradation rate of trans-isomer was slightly faster than that of cis-isomer. The authors concluded that the reason for the lower recovery of the total radio carbon of trans-permethrin was that the products derived from trans-isomer appeared to be more easily degraded to volatile products.

Figure 4. Degradation pathways for permethrin in soil[50,51]



Kaufman et al.[51] also studied the degradation of permethrin labelled at ^{14}C-carbonyl (acid) and ^{14}C-methylene (alcohol) in soil under laboratory conditions. Their investigation indicated that permethrin was rapidly degraded in soil with the half-life less than 28 days. Again, degradation of the trans-isomer occurred more rapidly than with the cis-isomer. From the analyses, Cl_2CA (18), PBalc (19), PBacid (21) and the parent material (8) appeared as the predominant radioproducts. Numerous other ^{14}C-products, presumably various hydroxylated parent compounds (23) as well as hydroxylated cleavage products (20) occurred in quantities less than 1% of the total products isolated. However, the degradation products and pathways of permethrin in the study were quite similar to those in the observations of Kaneko et al.[50]

Williams and Brown[52] examined the persistence of permethrin in a variety of soils at the laboratory. Soils were fortified with permethrin at 1 mg/kg and incubated for 16 weeks at temperatures alternating between 20°C for 15 hours and 10°C for 9 hours to simulate average day and night summer temperature. They found that in all tested soils, except one of them, permethrin degraded rapidly resulting in half-life of 3 weeks or less for both isomers. In the other soil, which was organic soil, with lower pH level the rate of degradation was slow; over 75% of cis-permethrin was recovered after 16 weeks treatment and slightly less for trans-isomer. The authors also determined the effect of soil sterilization on the degradation of the insecticide, observing that the degradation rate in the sterilized soil was greatly reduced; from which they concluded that microbial activity was the major factor in the loss of the insecticide.

Again, laboratory study on the persistence and behaviour of permethrin in a southern Ontario soil was carried out by Harris et al.[53] In their study, 100 g of Plainfield sand sample was treated with permethrin at the rate of 0.01 to 10 ppm, and 50% of the initial application was lost in 5 weeks. Trans-isomer declined more rapidly in the tested sand than the cis-isomer, which behaviour was also observed in all of the previous studies.[50-52] In comparison of rate of degradation with R- and S-configuration, the study reported that 1S, trans-isomer declined much more rapidly than the corresponding 1R isomer during the first 6 weeks. However, little difference was observed in the rates of degradation of 1S, cis- and 1R, cis-permethrin.

VII. Fate of permethrin in plants

The short-term metabolism of ^{14}C-permethrin in snap bean seedlings has been studied in the glasshouse.[54] Cis- and trans-permethrin isomers, labelled separately in the dichlorovinyl group and in the methylene carbon atoms, as shown in Figure 5, were used to treat individual bean leaves on 14-day-old seedlings at the rate of 15 µg per leaf. Autoradiography of plants prior to analysis showed that little translocation of radiolabelled permethrin or its metabolites had occurred.

They found some interconversion of cis- and trans-isomers occurred on the leaf by photochemical reaction. As in the soil studied, the cis-isomer was found to be a little more persistent than the trans-isomer and the initial half-lives were 9 and 7 days, respectively. In addition to the parent compound, a large number of metabolites were present in the plant

extracts, the most important ones from the alcohol moiety being PBalc (19), PBacid (21), and 2'-hydroxy (25) and 4'-hydroxy (26) derivatives of 3-phenoxybenzyl alcohol. In their studies, hydroxy substitutions on permethrin at 2'- and 4'- positions, (24) and (23) respectively, were also observed (see Fig. 7).

The cis- and trans-cyclopropane carboxylic acids (Cl_2CA, 18) were the major metabolites from the acid moiety and these were found mainly in the conjugated form. The conjugated acid and alcohol compounds were hydrolysed to free compounds by using either β-glucosidase or hydrochloric acid. It was inferred that the sugar concerned was glucose but no further detailed evidence of the identity was obtained.

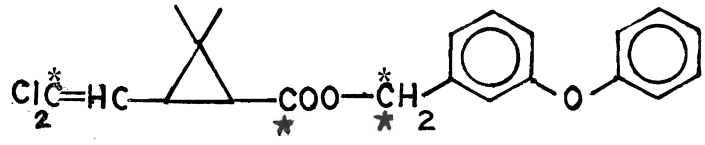


Figure 5. Structure of permethrin showing positions of ^{14}C-labelling [used by (*) Ohkawa et al.[54] and (★) Casida and Gaughan[57]]

Gatehouse et al.[55] studied the degradation of ^{14}C-permethrin on cotton leaves, cabbage leaves and apple fruits. In all cases, they found that permethrin degraded slowly; unchanged permethrin accounted for 23-58% of the radio activity on cotton leaves 28 days after application, more than 60% of the radioactivity on cabbage leaves 42 days after treatment and more than 80% of the radioactivity in apple fruits 28 days after

spraying. The above results indicated that the rate of degradation of permethrin was varied from one type of crop to another.

In a separate study, the same group[56] designed experiments to examine the possible uptake of permethrin and its degradation products by plants from soil. In their study, sugar beet, wheat, lettuce and cotton seeds were sown in soil treated with ^{14}C permethrin. Sandy loam soil was treated at a rate of 2 kg/ha with [^{14}C-cyclopropyl]-permethrin or [^{14}C-phenyl]-permethrin as shown in Figure 6.

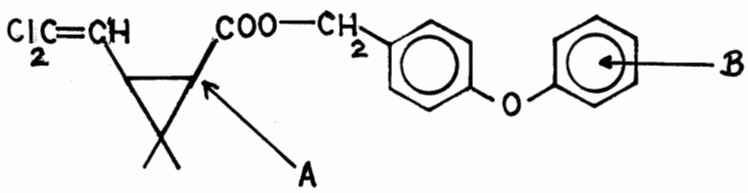


Figure 6. Positions of radiolabelling in two ^{14}C-permethrins; 'A' is labelled at cyclopropyl acid and 'B' is labelled at a phenyl group as shown.

The applied compound was thoroughly mixed with the top 8 cm of soil and seeds were sown 30, 60 and 120 days after treatment. In mature plants, from seed sown 30 days after soil treatment, low radioactive residues of up to 0.86 µg/g were detected. However, the transfer of radioactive residues into the crops decreased very significantly as the interval between soil treatment and sowing increased, so that with an interval of 120 days, it declined to 0.09 µg/g or less. In general, higher residues were found in crops grown in soil treated with [^{14}C-cyclopropyl]-permethrin and the metabolites derived from it were found to be cis- and

trans-Cl_2CA(18) and cyclopropane 1,2-dicarboxylic acid (22).

The authors concluded that these acid metabolites were formed in soil and were subsequently taken up by the plants, whereas in the parallel experiments with [^{14}C-phenyl]-permethrin considerably lower radioactive residues were present.

The most comprehensive study of the metabolism of permethrin was reported by Casida and Gaughan[57] in 1978. In their study with snap beans and cotton, four radiolabelled samples were used, namely cis- and trans-permethrin labelled separately in the carboxyl or methylene carbon atom as shown in Figure 5. In the greenhouse, individual leaves of snap beans and young cotton plants were treated topically with 1 μg of cis- or trans-permethrin labelled either in the carboxyl or methylene carbon. Similar treatments were made to cotton plants grown in an ordinary field in California. Stem injection of bean plants was used as an alternative application method. However, results from stem injection experiments with respect to terminal residues were less satisfactory than results from topical experiments.

Under outdoor conditions, the authors found that about 30% of the radioactivity was lost from the cotton plants after one week. The results of 3-week and 6-week cotton leaves showed that the cis- compounds gave higher recoveries than the trans-compounds. Again, in all experiments, it was confirmed that the trans-isomer degraded more rapidly than the cis-isomer.

The major degradation pathway included the cleavage of ester and the cleavage products, Cl_2CA (18) and PBalc (19) conjugated rapidly

with some plant constituents. The authors noted that not all the conjugates were readily cleaved by β-glucosidase, so it was inferred that these are likely to be compounds other than simple glucosides. However, they are not likely to be amino acid conjugates since they did not undergo methylation. Another possibility was that glucose esters of carboxylic acids were present but were less readily hydrolysed by β-glucosidase than the ether-linked glucosides.

Other identified products included those hydroxylated compounds, -OH substitution at 2' and 4' of the phenoxy group of the parent (24) (23) and alcohol metabolites (25) (26). In addition, oxidation at the methyl group of the acid moiety (20) had taken place followed by conjugation. It was interesting to note that the HO-Cl_2CA conjugates were found in larger amounts from cis-permethrin than from the trans-isomer; presumably this was due to the greater resistance of the ester group of the cis-isomer to hydrolysis allowing more time for oxidation prior to ester cleavage. They also observed photoisomerization at the cyclopropane on the leaves, i.e. interconversion of the trans- and cis-isomers.

In treated beans, the permethrin isomers underwent similar metabolism and conjugation except that the degradation rate in bean plants was about two fold more rapid than that in the cotton foliage under the greenhouse condition. No further explanation of this statement was given. However, this result indicated that although the experiments were carried out under identical conditions, the rate of degradation was, in part, based on the types of plants. The combined information on metabolism of permethrin in plants was summarized in Figure 7.

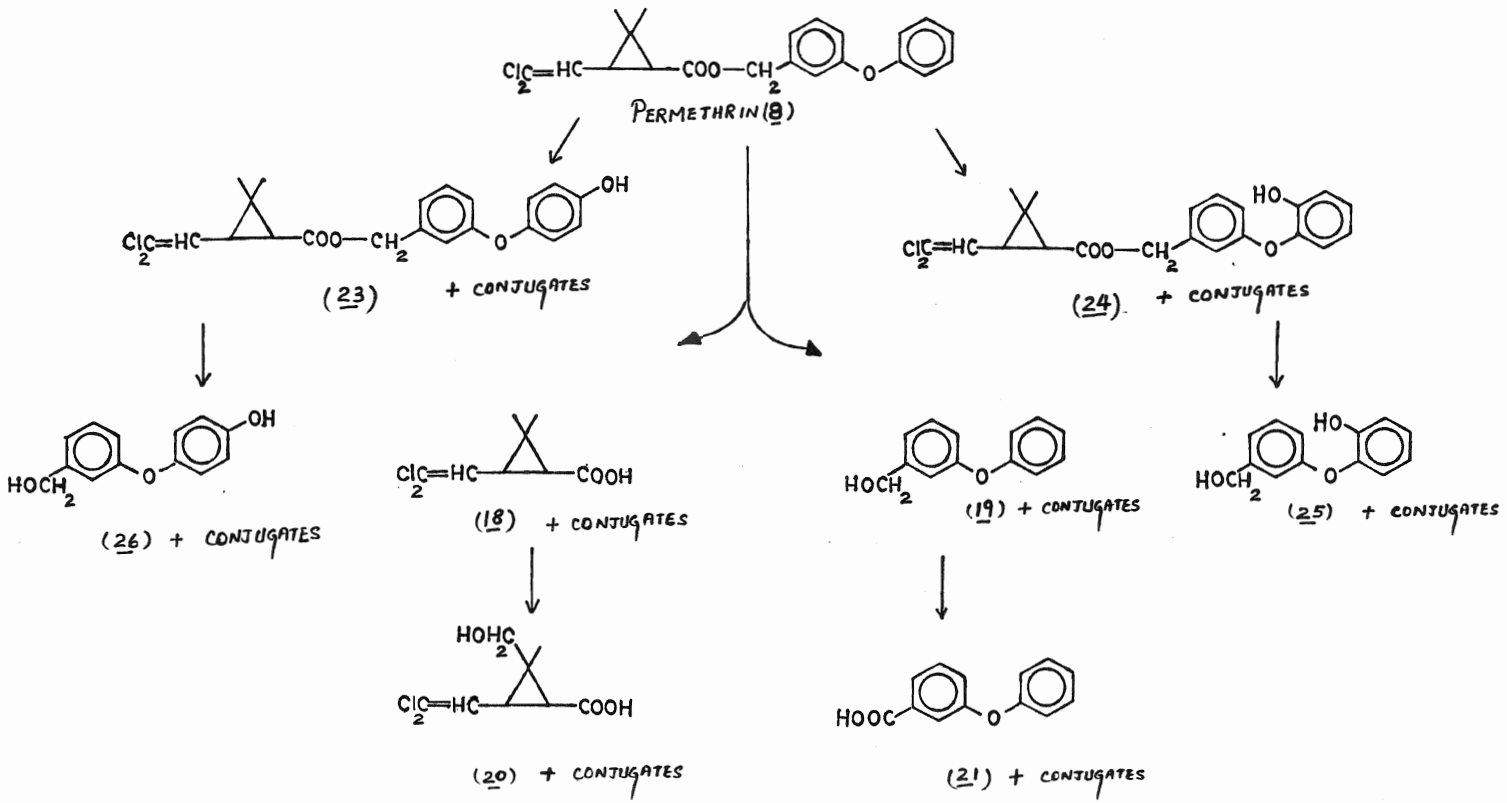
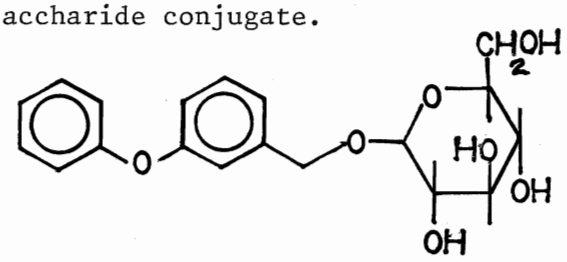


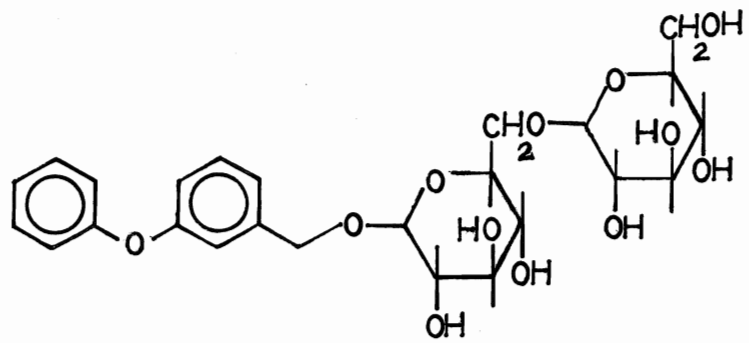
FIGURE. 7 METABOLISM OF PERMETHRIN IN PLANTS

Chiba[58] also studied permethrin isomers on peach foliage. In this study, permethrin was applied at 0.21 kg (AI)/ha as a 25% WP formulation to peach trees in early June and mid July. The study indicated that the rates of dissipation of both isomers were similar and the residues of permethrin 21 days after application in June and July were about 10% and 41% respectively of the initial deposits remaining on the leaf. Under identical environmental and experimental conditions, the lower recovery of permethrin in the June study was probably due to the result of rapid leaf growth. The half-life of permethrin on peach leaves was reported ranging from 7 to 10 days.

Roberts and Wright[59] studied the metabolism and conjugation of one of the major degradation products of permethrin, PBalc (19), labelled at alpha carbon atom, in cotton leaves from the plants which were grown in the glasshouse with natural daylight and artificial heat. In the study, they found that a ^{14}C-labelled compound was converted rapidly to glucosyl 3-phenoxybenzyl ether at the initial stage followed by forming the more polar disaccharide conjugate.



glycosyl 3-phenoxybenzyl ether



disaccharide conjugate

The experiments with [^{14}C]-glucose indicated that there was ready exchange of the glucose units on the conjugate with free glucose in the leaves with the half-life of a few hours.

The authors concluded that these ether-linked conjugates were relatively stable to hydrolysis by acid and alkali in comparison with the ester-linked conjugates. However, these ether-linked compounds were readily hydrolysed by enzyme systems *in vitro* and *in vivo*.

VIII. Fate of fenvalerate in soil

Williams and Brown[52] determined the persistence of fenvalerate in soil as residues from spray applications under laboratory conditions. The study was conducted with six different British Columbia soils having the pH range from 4 to 8 and various constituents. An individual soil sample was fortified at the rate of 1 mg/kg and stored in the dark at average day and night summer temperature. The study revealed that times for 50% disappearance of fenvalerate in 5 different soils were approximately 7 weeks, whereas the soil with high organic matter and low pH revealed a slower degradation rate; for example, more than 75% of fenvalerate was recovered 16 weeks after application.

Under laboratory conditions, Harris *et al.*[53] also studied the persistence and behaviour of fenvalerate in Plainfield sand. The study showed that the half-life of fenvalerate under tested conditions was 9 weeks and 12% of the initial application was recovered 48 weeks after treatment.

The same group, in a separate study,[60] examined the persistence of fenvalerate in a mineral (sand) soil and an organic (muck) soil. The study indicated that due to the differences in the density and moisture content in the mineral and organic soils, the initial concentration levels on the former were always lower than the latter, although both of them had received the same treatment. In assessing the persistence of fenvalerate in incorporated organic soil, the authors reported that 25%, 17% and 7% of the pesticide initially applied were still remaining after 6, 18 and 28 month-periods, respectively.

[^{14}C-carbonyl]- and [^{14}C-cyano]-fenvalerate were used to study the degradation of fenvalerate in several Japanese soils.[61] Each soil was treated at a rate of 1 mg/kg and stored at 25°C in the dark under aerobic conditions. The initial half-life for disappearance ranged from two to 12 weeks depending on the soil type, whereas under the same conditions in a separate study,[50] the authors reported that (+)-trans- and (+)-cis-permethrin were six to nine days and twelve days, respectively.

In this study, the degradation products formed from the labelled fenvalerates were analogous to those from permethrin; those products were formed as results of ester cleavage, ring hydroxylation in the 3-phenoxybenzyl moiety and hydrolysis at the cyano group (29). From the ester cleavage, an unstable cyanohydrin type intermediate (32) was produced which could easily be decomposed to the corresponding aldehyde (38) and H^{14}CN. However, in their study, H^{14}CN was not found at a detectable level under various test conditions. In contrast, an extensive $^{14}CO_2$ was found and the amounts of $^{14}CO_2$ generated were always greater

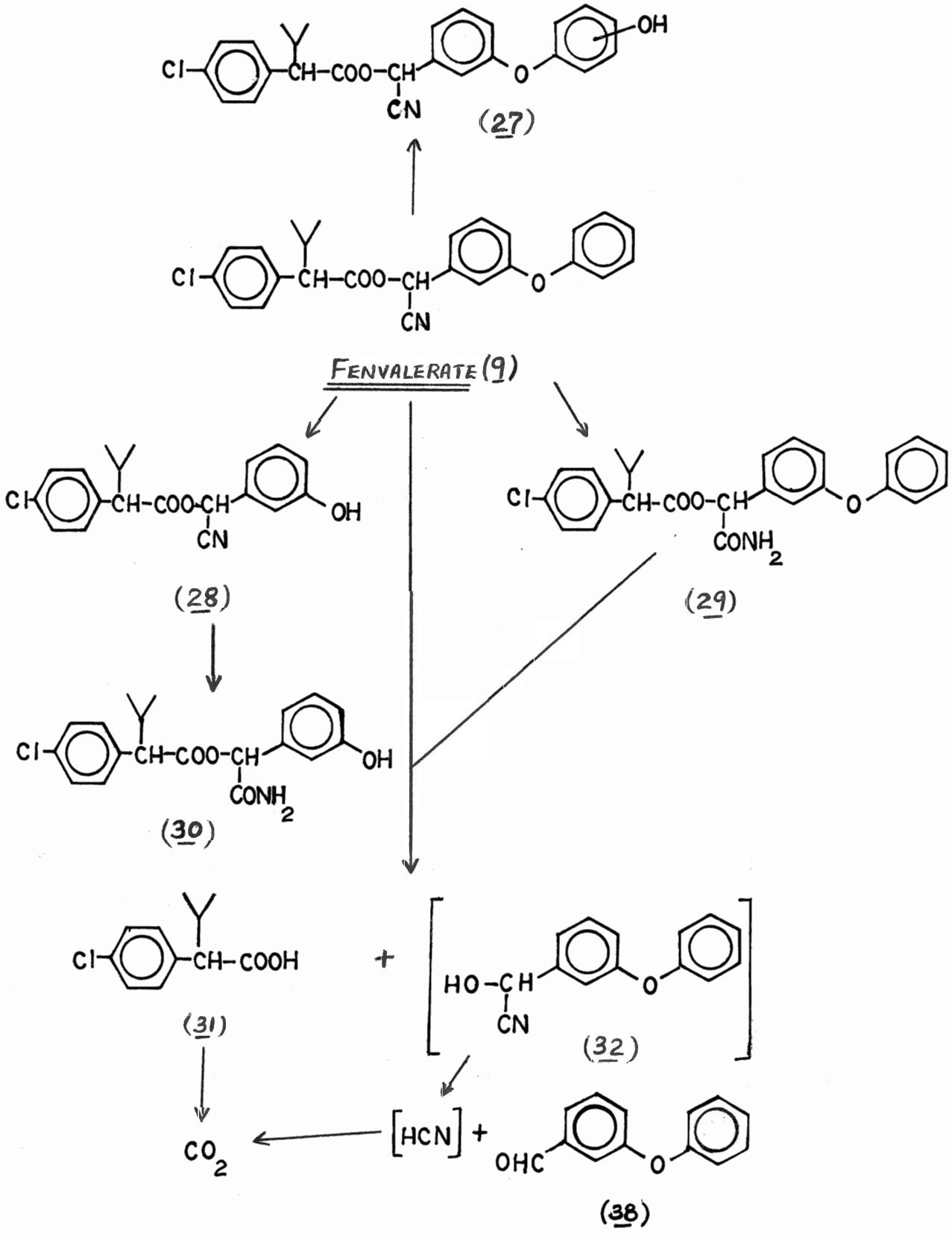
from [^{14}C-cyano]-fenvalerate than from [^{14}C-carboxyl]-fenvalerate.

Another degradation route, ether cleavage, was also observed and resulted in the formation of des-phenyl fenvalerate (28) which still has the ester linkage intact. The amount of this compound present ranged from 0.2 to 5.5% of the applied radioactivity in all tested soils after 30 days. This reaction did not occur with permethrin although it contains the diphenyl ether linkage in the alcohol moiety. However, the authors concluded that the ether cleavage had probably resulted from unstable 2'-hydroxy fenvalerate and no further clear explanation was given. The ether cleavage of the amide analogue (30)of fenvalerate (29) was also observed. For comparison with the study performed under anaerobic conditions, and the following observations were made: the degradation rate was much slower, no $^{14}CO_2$ was produced from [^{14}C-carboxyl]-fenvalerate, but approximately 10% of the applied [^{14}C-cyano]-fenvalerate was released as $^{14}CO_2$. A summary of fenvalerate degradation in soils from the study is given in Figure 8.

IX. Fate of fenvalerate in plants

There have been few published reports on the persistence of fenvalerate in various field crops. Greenberg[62] described a method for determining fenvalerate in field treated samples, i.e., grapes, pepper, apple and cottonseed which were grown in Israel. The study illustrated the half-life of sprayed deposits on grapes, peppers and apples were within 14 days. A similar study was reported by Talekar[63] on field-treated cabbage. During the study period, the half-life of fenvalerate

Figure 8. Degradation of fenvalerate in soil



under Asian weather conditions was less than a day. In another study, Harris et al.[64] applied fenvalerate at 0.14 kg/ha to celery grown in outdoor microplots. The residue levels they found were 3.3, 1.7, 0.45 and 0.36 ppm after 0, 7, 14 and 21 days, respectively.

Hill et al.[65] determined the persistence of fenvalerate residues in alfalfa under southern Alberta conditions. In their experiments, the compound was applied at 0.14-0.15 kg/ha and the initial residues of 22.2-32.5 ppm from three separate experiments in different months of application resulted in exponential decline with a half-life of 9-11 days.

Ohkawa et al.[66] studied the metabolic fate of fenvalerate in bean plants under laboratory conditions. The experiments were carried out with fenvalerate, labelled separately with ^{14}C at the benzylic, cyano and carbonyl carbon atoms and applied to the leaf surface individually at the rate of 10 µg per leaf. The initial half-life for disappearance of ^{14}C compounds was approximately 14 days. The authors observed that very little of ^{14}C was translocated to other parts of the plant.

Fenvalerate underwent metabolism in the plants by several routes and produced various polar metabolites. The major metabolites of alcohol constituents were PBald (38), PBalc (19), PBacid (21), 2'-OH-PBacid (36) and 4'-OH-PBacid (37), most of which were isolated as sugar conjugates. The minor conversion of the cyano group to the amide (29) was also detected. In addition, PBalc-COOH (35) was formed when polar material (34) was hydrolysed. The main product of the acid moiety was conjugated-CPIA (31). The decarboxy derivative (33) of fenvalerate was also

detected in leaf extracts, based on the studies reported by Holmstead et al.[67] and Mikami et al.;[68] the authors assumed that the decarboxy compound was probably formed by photochemical conversion on the leaf surface. The authors also reported that there were also unextractable bound residues in bean plants as major constituents of the residues which were cleaved very small extents by enzymatic hydrolysis. The summary of the metabolic pathways of fenvalerate in bean plants is shown in Figure 9.

X. Analytical methods used for determination of permethrin and fenvalerate

A number of analytical methods have been published for determination of residues of permethrin and fenvalerate in various crops. Most methods follow very similar procedures for measurements of the intact parent compounds as follows:

1. initial extraction into organic solvent
2. liquid-liquid partitions to remove water soluble coextractives
3. cleanup of the extracts by adsorption chromatography
4. quantitative determination of the insecticide residues largely by gas-liquid chromatographic methods.

In general, the tested crops or leaves were homogenized with either single non-polar solvent, e.g., hexane[68] and benzene,[58] or one of these solvents in combination with a more polar solvent, e.g., acetone[63,70] at initial extraction. Since the insecticides are also soluble in polar organic solvents, Lee et al.[71] used acetonitrile for extraction of

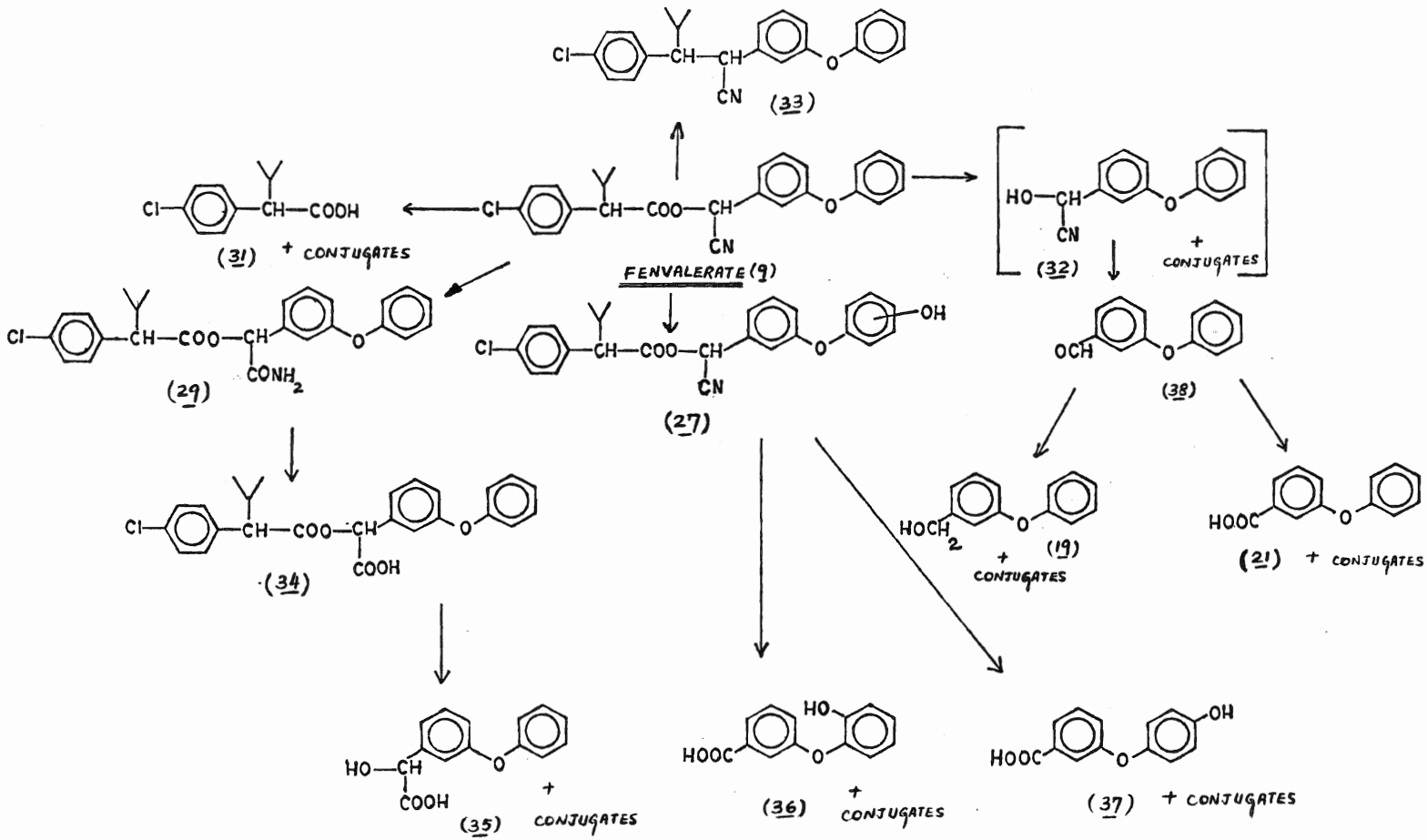


FIGURE. 9 METABOLISM OF FENVALERATE IN PLANTS

fenvalerate from cabbage and lettuce. Gaughan and Casida[57] utilized the mixture of methanol-chloroform for the extraction of permethrin residues on cotton and bean leaves.

Liquid-liquid partitions were performed with a variety of polar organic solvents along with water in order to remove polar organic materials and water soluble coextractives. For example, mixed solvent systems such as hexane-methanol-water,[69] hexane-acetone-water,[72] petroleum ether-1% sodium chloride solution-acetonitrile[71] and petroleum ether-saturated sodium chloride solution-acetone[62] systems were used for partitioning. Since various vegetables and other crops have different components and character, the selection of particular solvent systems for extracting and partitioning is varied widely.

Removal of interfering substances by adsorption chromatography was a typical cleanup procedure prior to quantitative determination. As to the adsorbents, Florisil, alumina and silica gel were used frequently with a variety of single and mixed solvents as eluents. The order of eluting system, in general was started with nonpolar solvent followed by gradually increasing the polarity of the solvent system. Chapman and Harris[72] reported that the use of Florisil with a mixture of hexane-benzene (20:80) afforded good cleanup for most crops studied.

As both permethrin and fenvalerate have electronegative chlorine atoms (two in permethrin, one in fenvalerate), gas-liquid chromatography with the use of electron capture detection (GC-ECD) was used almost exclusively for determination of both compounds mainly because of the superb sensitivity of this technique.

As an alternative to gas chromatographic methods, there were a few reports on high performance liquid chromatographic separation of the isomers of permethrin and fenvalerate. Kikta Jr. and Shierling[73] have developed HPLC methods for rapid analysis of technical permethrin on both normal and reversed phase columns. The authors reported that the use of normal phase system with a UV-detector operated at 220 nm gave the minimum detectable concentration of 40 ppb of permethrin (w/w), deposited on cotton leaves without any prior cleanup.

In another study, Mourot et al.[74] developed a method for analysing both permethrin and fenvalerate by HPLC. In their study, the isomers of both compounds were well-resolved on a Lichromosorb Si-60 column with a UV-detector operated at 235 nm; a mixed solvent system consisting of hexane-isopropyl ether (90:10) was used as a mobile phase. However, the study was performed only at a high concentration range, i.e., 1 gram of each compound in one litre chloroform, and made no attempt to determine at residue levels.

To date, studies on metabolism of permethrin[54,57] and fenvalerate[66] were performed only with ^{14}C-labelled compounds. No publications were found in the open literature describing the studies conducted to investigate the breakdown products of these unlabelled insecticides under natural conditions.

In the studies of ^{14}C-labelled compounds, the treated leaves were either freeze-dried and ground to a fine powder,[54,66] or cut into small pieces[67] prior to extraction with polar organic solvents. Then the parent compounds and their metabolites were separated and identified by thin layer chromatography and the radioactivity in the extracts was

determined by liquid scintillation counting. Unextracted residues were analysed by combustion, and radioactive spots on TLC were detected by radioautography. The studies also proved the presence of glucoside conjugated products. The identification of the metabolites after cleavage of the conjugated products was made by TLC (both one- and two-dimensional), comparing R_f values with authentic standards.

XI. Purpose of this study

Permethrin and fenvalerate were developed in the early 1970's, and are now being used widely in many countries. In Canada, however, only permethrin was registered in 1978 for limited use and its use was extended in 1979. Fenvalerate was registered in 1980 on a temporary basis for limited use; this condition has not been changed to date. The knowledge of their effectiveness against various insects, toxicities to mammals, and their photostability under natural and simulated sunlight were extensively studied in the past few years. Although there is now abundant evidence that these compounds have outstanding characteristics, it is still important to know precisely their persistence, and the nature and toxicity of their metabolites that are produced under different environmental conditions.

Though a few analytical reports have been published from all over the world on determination of their persistence in various crops with different environmental conditions, to date there are only a handful of papers published, in which metabolism or degradation of these compounds were discussed, and most studies were done either in California or in

Japan. Moreover, all of these metabolic studies were conducted with ^{14}C-labelled compounds and were mostly performed under laboratory conditions. No study was carried out with non-radioactive compounds under natural orchard conditions; naturally no study has been done in Canada to date.

The purpose of this study was to determine the persistence of both permethrin and fenvalerate residues in apple foliage under environmental conditions of Niagara Peninsula, Ontario, and also to identify their degradation compounds.

Since both permethrin and fenvalerate have electronegative chlorine atoms in the molecules (one in fenvalerate, two in permethrin), the persistence and the fate of these insecticides in the leaves could easily be determined by GC-ECD. For identification of possible degradation compounds, high performance liquid chromatography (HPLC), mass spectrometry (MS) and gas chromatography coupled with mass spectrometry (GC-MS) could be used.

The results of this study will provide firsthand information regarding the fate of these compounds, under Canadian climatic conditions; the information may be used when making further recommendations as to the use of these chemicals and elucidating ecological complications which are being created as the result of using these chemicals.

Experimental

I. Materials and Reagents

(1) Pesticide standards

Analytical standards of cis- and trans-permethrin (3-phenoxybenzyl-(±)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, 99% purity) were provided by FMC Corporation, Middleport, New York, U.S.A. 14105, and a commercial formulation, 25% wettable powder (cis:trans ≅ 40:60) was supplied by Chipman Chemical Limited, Stoney Creek, Ontario. Analytical grade 3-phenoxybenzyl alcohol (PBalc, 19), 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (cis:trans ≅ 40:60), cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (c-Cl_2CA, 18) and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (t-Cl_2CA, 18) were obtained from Imperial Chemical Industries Limited, Bracknell, Berkshire, U. K.

Technical grade fenvalerate (9) [α-cyano-3-phenoxybenzyl-2-(4-chlorophenyl)-isovalerate] was supplied by Shell Canada, Chemical Company, Toronto, Ontario and it was used as the standard in the study. The probable degradation compounds of fenvalerate, 3-phenoxybenzaldehyde (PBald, 38) and 2-(4-chlorophenyl)-isovaleric acid (CPIA, 31) were not available during the study, it was thus decided to prepare them by base hydrolysis of fenvalerate. Several studies[61,75,76] reported that under ambient conditions, PBald (38) was formed from inherently unstable cyanohydrin intermediate (32), which was the initial hydrolytic product of

fenvalerate. With the following procedure of base hydrolysis of fenvalerate, PBald (38) and CPIA (31) were obtained. To ten grams of fenvalerate in 100 mL methanol, 20 mL of 20% NaOH solution was added dropwise (pH ≅ 14). Then the mixture was refluxed at room temperature for 6 hours and separated by partitioning with 100 mL of hexane and 600 mL of water. The aqueous layer was shaken twice with 100 mL of hexane. The combined hexane extracts were dried over anhydrous sodium sulphate. The pale yellow liquid product, PBald (38), was obtained by evaporating the hexane. The aqueous layer was then acidified with sulphuric acid to pH ≅ 1.2 and treated three times with 100 mL of ether. The ether extracts were combined and dried over anhydrous sodium sulphate. The yellowish solid product, CPIA (31), was obtained after removal of ether. The alcohol moiety of fenvalerate, PBald (38), was characterized by GC with a flame ionization detector and by GC/MS, which is shown in Figure 24. The acid product, CPIA (31), was methylated with diazomethane and was characterized by GC/MS as shown in Figure 28.

2. Solvents

(a) Pesticide grade solvents Hexane and acetone were used for all the leaf extractions, liquid-liquid partitions and preparation of stock sample solutions for gas chromatographic studies.

(b) Spectrograde solvents Acetonitrile, hexane and methylene chloride were used for high performance liquid chromatographic (HPLC) analyses. Methanol was used for extraction of silica gel from thin layer chromatographic (TLC) studies and in the reaction of methylation.

(c) ACS reagent grade Ether was used for the extracting purposes.

All the aforementioned solvents were obtained from Caledon Laboratories Ltd., Georgetown, Ontario.

3. Reagents and Chemicals

The following list gives the grades and suppliers of the reagents and chemicals used.

(a) Anhydrous sodium sulphate (Certified ACS grade, Fisher Scientific Company, Fairlawn, New Jersey).

(b) Anhydrous potassium fluoride (Laboratory Reagent, BDH Chemical Ltd., Poole, England).

(c) Glycerol (Laboratory Reagent, BDH Chemical Ltd., Poole, England).

(d) Diazald® (N-methyl-N-nitroso-p-toluene sulfonamide) (99% purity, Aldrich Chemical Company, Indiana).

(e) α-bromoacetophenone (98% purity, Aldrich Chemical Company, Inc.)

(f) Carbon disulphide (ACS grade, J. T. Baker Chemical Co., Philipsburg, New Jersey).

(g) Dimethylformamide (distilled in glass, reagent grade, Burdick and Jackson Laboratories, Inc., Muskegon, Michigan 49442).

(h) t-Butyldimethylchlorosilane/imidazole reagent (Applied Science Laboratories, Inc., State College, Pennsylvania 16801)

(i) Trichloroethanol (Eastman Kodak Company, Cat. No. 6380)

(j) Trifluoroacetic anhydride (Eastman Kodak Company, Cat. No. 7386).

(k) Florisil (ACS grade, 60-100 mesh, activated, Fisher Scientific Company).

II. Small apparatus

The following apparatus were used during the studies.

1. Fisher-Kendall Mixer, Fisher Scientific Company, Don Mills, Ontario.
2. Polytron homogenizer, Kinematics GmbH, Switzerland.
3. Diazald® kit for the safe preparation of diazomethane.

III. Instruments for determination and their operating conditions

1. High performance liquid chromatograph (HPLC)

(a) A Spectra-Physics model SP-8000 liquid chromatograph equipped with a data system was used. A SP-8300 UV-Vis detector with an appropriate lamp (standard mercury vapour lamp) and filter was operated at 254 nm. The temperature of the columns was maintained at 30°C. The instrument was also equipped with a 6000 psi delivery system and constant flow capability. For the collection of sample, a 100 microlitre (μL) loop, and for analysing collected samples, a 25 μL loop were used.

(b) An HPLC system, composed of Milton Roy minipump (5000 psi), a Rheodyne model 70-10 injector, a 100 μL sample loop and a Spectra-Physics model SP-8200 UV-Vis detector at 254 nm, was operated. The recorder used was a Varian Aerograph model 20.

The following columns were used in the above HPLC systems.

(i) Brownlee Labs RP-18 10 μm 4.6 mm I.D. x 25 cm

(ii) Whatman Partisil PXS ODS 5 μm 4.6 mm I.D. x 25 cm

(iii) Zorbax ODS 4.6 mm I.D. x 15 cm

(iv) Zorbax ODS Semi-preparative 9.4 mm I.D. x 25 cm

(v) Zorbax CN Semi-preparative 9.4 mm I.D. x 25 cm

2. Gas Chromatograph

(a) With an Electron Capture Detector (GC-ECD)

A Varian Aerograph model 328 with a ^{3}H (tritium) electron capture detector and Fisher Recordall® series 5000 recorder were used. In this study, titanium tritide (250 mCi) or scandium tritide (1 Ci) foil was used as a source of radioactivity and is essential in this detector. The operating temperature for the titanium foil is limited to 220°C and for the other is up to 325°C in order to prevent the loss of radioactivity. The electron capture detector is extremely sensitive to certain molecules such as halides, conjugated carbonyls, nitriles, nitrates and organometals, but is virtually insensitive to hydrocarbons, alcohols, ketones etc.[77]

The operating principles of the detector were as follows. As the nitrogen carrier gas flows through the detector, a tritium source ionizes the nitrogen molecules and slow electrons are formed. These slow electrons migrate to the anode under the fixed voltage and produce a steady current which is then amplified by the electrometer. If a compound containing an electron absorbing group is introduced to the detector, it will cause the reduction of this current. The loss of current is a measure of the amount and electron affinity of the compound.

The following columns were used in the studies.

(i) 0.9 m x 2.4 mm I.D. glass column packed with 3% Silar-9CP on Gas Chrom Q 80-100 mesh.

(ii) 0.9 m x 2.4 mm I.D. glass column packed with 3% OV-275 on Chromosorb W-HP 80-100 mesh.

(b) With a flame ionization detector (GC-FID)

A Varian Aerograph model 550 equipped with a Varian flame ionization detector was used along with a Varian Aerograph model 20 recorder. The flame ionization detector responds to almost all compounds except water, carbon disulphide and air. Since carbon disulphide does not respond to FID and dissolves PBalc (19) and PBald (38) well, it was used as the solvent for all the analyses which were conducted with GC-FID.

The operating principle of FID was as follows.[77] The effluent gas from the column was mixed with hydrogen and burned with air or oxygen in the detector chamber. Then ions and electrons formed in the flame were passed through the electrode gap and decrease the gap resistance. However, the increase in the number of charged particles in a sample resulted in a decrease of the gap resistance. This decrease permitted current to flow in the external circuit which produces a signal and registers as a peak on the recorder.

The column used in the analyses was a 0.9 m x 2.4 mm I.D. glass column packed with 3% OV-275 on Chromosorb W-HP 80-100 mesh.

(3) Mass Spectrometer (MS)

The mass spectrometer used in the study was an AEI MS-30 double beam equipped with a Kratos DS-55 data system. For analysing solid substances, the sample was placed in a small glass cup at the end of a heatable probe which was inserted directly into the evacuated ion source via a vacuum lock. The sample was then evaporated by heating the probe in the temperature range 50-200°C. For liquid products, samples were introduced into an all glass

heated (200°C) inlet system (AGHIS), by a micro syringe through a silicone rubber septum.

The vaporized sample was bombarded with a 70 eV electron beam in the ion source where ionization and fragmentation occurred and produced a variety of different ions. A potential of 4 kV was used to accelerate the resulting ions firstly into a 90° electrostatic analyzer followed by a 90° magnetic sector, with a maximum mass of 650 a.m.u. at full accelerating voltage. Ions emerging from the magnetic sector were detected via an electron multiplier, amplified and the resulting signal was fed to the data system.

The data system then converted the analogue output of the mass spectrometer to digital form (20 bit), and the digital data were transformed into time centroid and intensity data for each peak. The requirement for the conversion of peak position data into mass data was to establish a calibration. The calibration is a correlation between the time and mass of peaks of known composition from a standard compound such as perfluorokerosene (PFK).

For the calibration, 1.0 μL of PFK was injected to AGHIS at 225°C, the source was tuned on the most intense peak (m/z = 181), a typical calibration range was 30-518 mass units. Then the collector intensity was set at an optimum, the operating resolution was about 1000, and data were collected. In order to stabilize the zero offset, 15-25 scans were initially acquired. The collected PFK time files data were converted to masses by the computer.

The nominal masses of the sample peaks were determined computationally from the mass/time scale established from the calibration,

using oxygen in the background as "lock mass". The mass spectrum was produced as a graphical report in which the relative intensity was plotted against mass for all masses in a given range. The graphical report was obtained through video display unit (VDU; HP 2649C) and the printer/plotter (HP 26319), whereas the quantitative data, a listing of the peak number, measured mass, absolute intensity, percentage of base intensity and total ion current (TIC), were printed from the printer terminal (D.G. DASHER).

(4) Gas chromatograph coupled with mass spectrometer

A Scientific Glass Engineering (SGE) all-glass single stage molecular jet separator or a Kratos Biemann frit separator was used to interface a Pye-Unicam Series 104 Gas Chromatograph and AEI MS-30 double beam mass spectrometer.

During the studies, small quantities of the sample solutions were introduced onto the gas liquid chromatographic column which was heated to the desired temperature. The solvent which was eluted from the column prior to the sample was vented to the atmosphere and the eluents were passed through the heated separator into the ion chamber through a vacuum lock system.

Since the scans were taken at regular and frequent intervals in time (e.g., four seconds per scan), the graphical report of total ion current (obtained by computer summation of all peaks) versus scan numbers and retention times for a series of scans provided a reasonable sample intensity profile. It was equivalent to a normal gas chromatogram, and was displayed

on the VDU. At the same time, quantitative data which included the measured masses and intensities were printed by a printer terminal. Then the mass data of high intensity peaks were plotted as mass spectra at a given mass range through the VDU and printer/plotter.

Peak averaging could be used to obtain more accurate mass and intensity measurement of weak peaks. However, peak averaging was a post-collection application which averaged the masses and intensities of peaks in two or more scans. When the background pressure was too high due to the liquid phase bleeding from the column or impurities, the subtraction of the intensities of the background from the sample was made in order to eliminate unnecessary peaks.

The following columns and conditions were used in the studies.

Columns:

(a) 1.2 m x 6.4 mm I.D. and 1.5 m x 6.4 mm I.D. glass columns were packed with 3% OV-275 on Chromosorb W-HP 80-100 mesh.

(b) 1.8 m x 6.4 mm I.D. glass column was packed with 5% QF-1 on Gas Chrom Q 60-80 mesh.

(c) 0.9 m x 6.4 mm I.D. glass column was packed with 3% SE-30 on Chromosorb W-HP 80-100 mesh.

Conditions:

Injector temperature: 210-220°C; separator temperature: 230°C; carrier gas: helium at 30-35 mL/min , oven temperature:50-220°C, detail will be reported individually in each study.

IV. Sample collections, preparations, extractions, fractionations of residues and cleanup

(1) Collections and preparations of the samples

Mature apple trees (cultivar McIntosh) were sprayed individually on July 7, 1980 with (a) permethrin (25% wettable powder) at 0.21 kg(AI)/ha and (b) fenvalerate (30% emulsifiable concentrates) at 0.14 kg(AI)/ha in an experimental orchard, Agriculture Canada, Vineland Station, Ontario. At each sampling date, 0, 23, 84 days after application, well exposed leaves were collected randomly throughout the plot. Each sample, consisting of two hundred and fifty grams of leaves without petioles, was kept in a glass jar and stored at -15°C until ready for extraction. The control (untreated) sample leaves were collected and prepared in the same manner as the treated samples.

(2) Extractions

Each of the leaf samples prepared as above was blended with a polytron homogenizer for 30 minutes with 1120 mL of a mixed solvent consisting of acetone and hexane (67% and 33% v/v) after the leaves had reached room temperature. One hundred and fifty grams of hyflo-supercel celite was added to the mixture which was filtered with suction using a Buchner funnel and Whatman No. 1 filter paper. The jar was rinsed two times with 100 mL of the acetone-hexane mixture which was added to the leaf residue in the funnel. Then the acetone-hexane extract was evaporated to about 250 mL with a rotary evaporator on a water bath at 50°C.

(3) Fractionations of residues

The above acetone-hexane extract was transferred to a separatory funnel and 100 mL of hexane and 50 mL of water were added. The mixture was shaken for 1 minute and the layers were allowed to separate. The upper hexane layer was expected to contain the parent compound (8 or 9) and some degradation compounds. In the case of permethrin treated sample, the most likely compound expected to be present in this fraction was PBalc (19). The lower acetone-water phase was re-extracted twice with 100 mL of hexane to ensure the extraction, and all the hexane extracts were combined and dried over anhydrous sodium sulphate. Then the dried hexane extract was evaporated to the volume of 250 mL (this fraction would be denoted as fraction No. 1 in the following discussion), and it was ready for analysing the parent compound (8) by GC-ECD and expected "free" alcohol, (PBalc (19), as a result of hydrolysis of permethrin), by GC-FID.

The aqueous phase was acidified with sulphuric acid to pH ≅ 1.2 and extracted three times with 100 mL of ether. In the same manner as the hexane extract, the ether extract was combined, dried and evaporated to the volume of 250 mL; this part (denoted as fraction No. 2) was expected to contain "free" Cl_2CA (18) as a result of hydrolysis of permethrin.

The aqueous layer after ether extraction, in which the "conjugated" Cl_2CA and "conjugated" PBalc were expected to be present, was concentrated under vacuum at 40°C to 200 mL. For the conjugated cleavage process, 200 mL of 12 N HCl was added to the concentrated aqueous layer and the mixture was heated at 70°C for 24 hours. After the treatment, the aqueous layer was shaken three times with 100 mL ether. The combined ether layer

was treated in the same manner as above; this part (denoted as fraction No. 3) was expected to contain Cl_2CA (18) and PBalc (19), which were produced from the cleavage of "conjugated" Cl_2CA and "conjugated" PBalc respectively.

Exactly the same procedures were taken for fractionations of fenvalerate and its degradation compounds; the major degradation compounds expected are CPIA (31) and PBald (38).

(4) Cleanup

(a) Column chromatography

One hundred grams of Florisil was deactivated by adding 5 mL of distilled water dropwise and tumbled for 30 minutes with a Fisher-Kendall mixer. Fifty grams of deactivated Florisil, prepared in the hexane as a slurry, was poured into a glass column (19 cm x 25 mm I.D.) which was fitted with a coarse fritted glass disk at the bottom. Forty grams of anhydrous sodium sulphate was added on top of the Florisil and tapped gently to settle the contents and was washed with 100 mL of hexane. The following samples were separated with the Florisil column thus prepared:

(i) Untreated sample (Control sample)

Two hundred mL of hexane extract (fraction No. 1, Section IV.3, two hundred gram leaf equivalent) was concentrated to 40 mL.

(ii) Untreated sample plus known quantity of standards

The spiked control sample was composed with the following:

(1) Two hundred mL of untreated hexane extract (fraction No. 1, Section IV.3, two hundred gram leaf equivalent)

(2) 2 mL of 200 ppm permethrin (25% wettable powder) in hexane

(3) 5 mL of 1275 ppm analytical stantard PBalc (19) in hexane

The above mixture was concentrated to 40 mL.

(iii) Treated sample

Two hundred mL of hexane extract from 84 day permethrin treated sample (fraction No. 1, Section IV.3, two hundred gram leaf equivalent) was concentrated to 40 mL.

Each of the above concentrated solutions prepared was added slowly onto the column right after all the washed hexane solvent was passed through the top of the packing material. Then the column was eluted first with 100 mL of hexane and followed by mixed solvents consisting of acetone and hexane with increasing percentage of acetone at 0.2, 0.5, 1.0, 2.0, 5.0, 8.0, 12.0 and 20.0 v/v % in hexane. Thirteen fractions with 100 mL in each fraction were collected and analysed by GC-ECD and GC-FID.

(b) Thin layer chromatography (TLC)

Silica gel plates used in the studies were Analtech Uniplate precoated silica gel GF chromatoplates without fluorescent indicator (20 x 20 cm, 2000 microns thickness, Mandel Scientific Company, Rockwood, Ontario) for preparative isolations and precoated 13181 silica gel No. 6060 chromatoplates with fluorescent indicator (20 x 20 cm, 0.25 mm thickness, Eastman Kodak Company, Rochester, N. Y.) for analytical purpose. In all the studies, the plates were developed two-dimensionally with the mixed solvent system, chloroform saturated with formic acid/ether (10/3, v/v).

For the preliminary study, authentic samples, PBalc (19) and Cl_2CA (18) were spotted on the fluorescent silica gel plate and developed with the mixed solvent system. Then the positions of the samples were detected under a UV light and measured the R_f values of PBalc (19) and Cl_2CA (18), which were found to be 0.7 and 0.9 respectively.

With the same procedures, the conjugated cleavage products (fraction No. 3, Section IV.3) of treated permethrin leaf samples as well as control sample were spotted on individual preparative TLC plates in order to cleanup the plant co-extractives prior to the analyses with MS and GLC. Based on the R_f values obtained from the preliminary study, the silica gel was scraped off from the area where PBalc (19) and Cl_2CA (18) were expected to be present and extracted firstly three times with 15 mL of carbon disulphide; this extract was expected to contain PBalc (19). Prior to analysis with GC-FID, the carbon disulphide extract was concentreated to 1 mL which contained 1 g equivalent of leaf extract.

The scraped silica gel was again extracted three times with 15 mL of methanol; in this extract Cl_2CA (18) was expected to be present. The methanol was evaporated to dryness under a gentle air stream and the residue thus obtained was analysed with the mass spectrometer using a direct inserting probe.

The conjugated cleavage products of fenvalerate sprayed samples were also treated in the same manner as mentioned above.

(c) High Performance liquid chromatography (HPLC)

The hexane extracts of permethrin (8) and fenvalerate (9) treated samples from fraction No. 1 (section IV.3) were studied with SP-8000 HPLC to investigate the presence of free alcohols, PBalc (19) and PBald (38). The extracts were separated and analysed by using various columns with different mobile phase compositions.

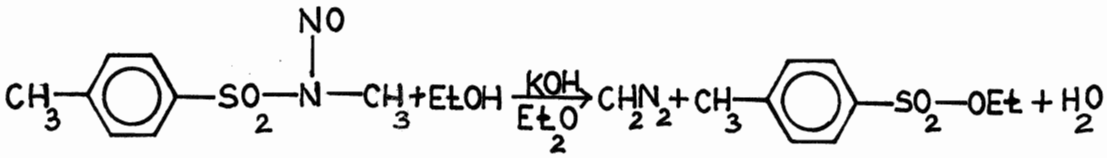
The study was first conducted by using the following individual reverse phase columns such as Brownlee Labs RP-18, Partisil PXS ODS, Zorbax ODS (regular and semipreparative) columns with various compositions of acetonitrile/water mobile phase. A method developed by McGarvey[78] was also studied with the above samples; in this method, two columns were connected via a switching valve. On the first column, the preliminary separation and cleanup of the plant coextractives occurred, and on the second column, the metabolite fraction was collected and analysed. Fractions where the alcohol constituent was expected to appear were also collected, concentrated and analysed on a different column.

An attempt was also made to analyze those samples by normal phase HPLC using a Zorbax CN semipreparative column with a mobile phase consisting of 80% hexane/20% methylene chloride.

V. Derivatization of the acid constituents

1. Derivatization with diazomethane

For the safe preparation of diazomethane, the Diazald kit was used and the same experimental set up illustrated in the Aldrich Catalog[79] was followed.



The procedure of diazomethane synthesis was as follows;[79,80] in a 500 mL round-bottom flask were placed 5 g potassium hydroxide in 8 mL of water and 25 mL of 95% ethanol. In a 125 mL separatory funnel, a solution of N-methyl-N-nitroso-p-toluenesulfonamide (Diazald®, 0.1 mole; 21.5 g) was dissolved in 200 mL of ether. The round-bottom flask was heated in a water bath at 65°C and the Diazald solution was added dropwise from the separatory funnel over a period of 30 min. As soon as all the solution had been added, additional ether (about 40 mL) was placed in the separatory funnel until the distillate coming out of the condenser was colorless. In the distillate, about 3 g of diazomethane was expected to be present and it was used immediately for derivatization of the standard acid constituents, cis and trans-Cl_2CA (18), and CPIA (31).

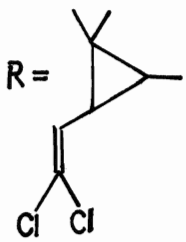
To complete the methylation of 1.1 mmole each of the aforementioned acid constituents, 2.0 mmole of diazomethane in 10% methanol/ether was required. These methylated esters formed were then analysed by GC-MS and GC-ECD.

(2) Derivatization with α-bromoacetophenone in the presence of potassium fluoride

The acid constituents such as the analytical standard of Cl_2CA (18) cis:trans ≅ 40:60), and the acid products obtained from base hydrolysis of permethrin (25% wettable powder) and fenvalerate (technical product) were

used for esterification with α-bromoacetophenone in the presence of potassium fluoride.

The procedure of the derivatization with α-bromoacetophenone was as follows.[81] Each of the acid products (1×10^{-3} mmole) in dimethyl formamide was allowed to react with an equimolar quantity of α-bromoacetophenone and approximately twice the acid concentration of potassium fluoride. The following reaction was accomplished at room



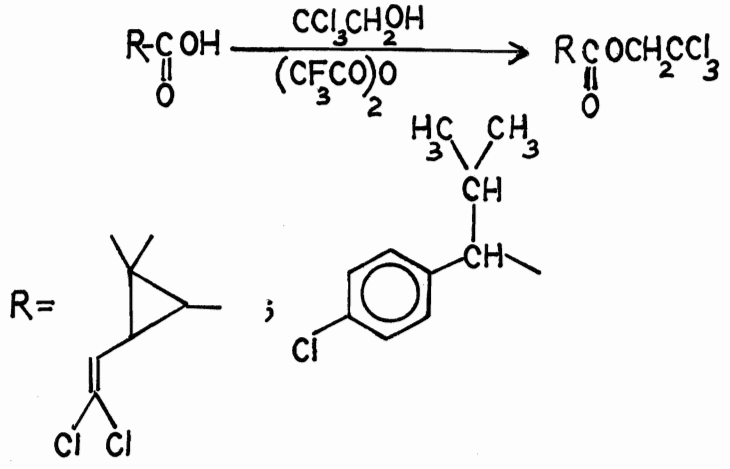
temperature in 30 minutes with efficient stirring of the mixture. Then the reaction mixture was filtered and an aliquot was taken and diluted with a solvent mixture consisting of 60% acetonitrile/water which was used as the mobile phase for HPLC analyses. A similar reaction was carried out in the absence of the acid component in order to identify the background of reagents on the chromatogram.

For the derivatization of actual sample (84 day permethrin treated) as well as control sample, 20 g leaf equivalent from fractions No. 2 and No. 3 (Section IV.3) in 10 mL extract was allowed to react with 1.3×10^{-3} mmole of α-bromoacetophenone in the presence of 2.1×10^{-3} mmole potassium fluoride. The reaction mixtures were then treated in the same

manner as the above reference compound and the esters expected to be formed were analysed with the Milton Roy HPLC system.

(3) Derivatization with the mixture of trichloroethanol and trifluoroacetic anhydride

The analytical standard of Cl_2CA (18) (cis:trans ≅ 40:60) and the acid products obtained from base hydrolysis of permethrin (25% wettable powder) and fenvalerate (technical product) were esterified with the mixture of trichloroethanol and trifluoroacetic anhydride. The reaction expected is shown below.



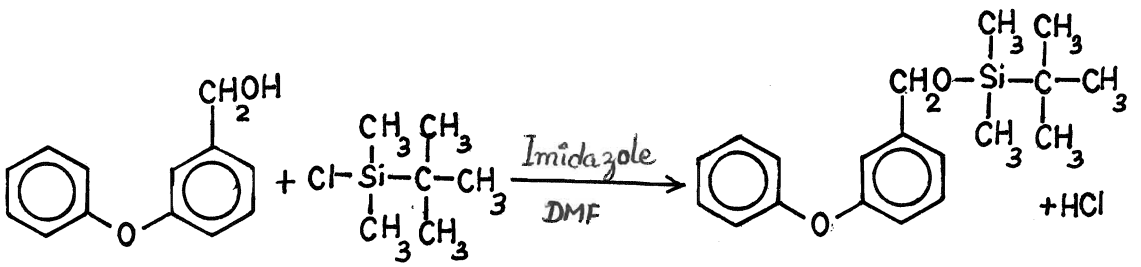
The method of derivatization, which was studied by Ohnishi et al.[75] was as follows. Each of the aforementioned acid products, 9×10^{-5} mole, was mixed with 0.2 mL of trichloroethanol and 0.5 mL of trifluoroacetic anhydride and the mixture was heated at 70°C for 20 minutes. Then to the reaction mixture, 10 mL each of hexane and water were added. The hexane layer was collected and the extraction was repeated two more times

with hexane. All the hexane extracts were combined and dried over sodium sulphate; the dried hexane extract was ready for GC-ECD analysis.

Similarly, control (with no acid) and 84 day permethrin and fenvalerate treated leaf samples (20 g leaf equivalent from fractions No. 2 and No. 3 (Section IV.3)) were treated as above and the reaction products were analyzed by GC-ECD.

VI. Derivatization of alcohol constituent of permethrin with t-butyldimethylchlorosilane/imidazole reagent

The analytical standard PBalc (19) was derivatized with a silane reagent by the following procedure.[82]



PBalc (19) 0.8 mmole was added to 1 mL of dimethylformamide solution which contained 1.0 mmole of t-butyldimethylchlorosilane and 2.5 mmole of imidazole. In order to ensure complete conversion from alcohol to silyl derivative, the mixture was heated to 160°C for 10 min. The resulting reaction solution was then extracted three times with 15 mL of ether in the presence of 40 mL of water. The ether layers were combined, dried over magnesium sulphate and evaporated to almost dryness with gentle air stream. Then the concentrated reaction product was prepared in adequate concentrations with hexane and analysed by GC-MS.

Results and Discussion

I. Determination of optimum flow rate

Since the effectiveness of chromatographic performance is based on the carrier gas flow rate, it is necessary to determine the optimum flow rate for the gas chromatograph at constant column temperature with various flow rates. For the study, permethrin (25% wettable powder) was introduced to GC-ECD and analysed on a 3% Silar-9CP column with the constant column temperature at 210°C. The number of theoretical plates, N, was calculated from the resultant chromatograms by measuring the retention time of the peaks and the peak widths. Based on the calculated number of theoretical plates, the height equivalent to a theoretical plate (HETP) was determined by the following equation:

$$HETP = \frac{L}{N}$$

where L = length of chromatographic column

N = number of theoretical plates

The value of N is given by the following equation

$$N = 16\left\{\frac{R_t}{w}\right\}^2$$

where R_t = retention time of the peak (measured in cm)

w = peak width (measured in cm)

The optimum flow rate could easily be determined by making a simple Van Deemter plot of HETP versus the gas flow rate. The most efficient flow rate corresponds to the minimum HETP, or maximum number of theoretical plates.[77] The results of the analysed sample at the concentration 7.5 µg/mL indicated that the optimum chromatographic performance could be obtained at a flow rate of 30 mL/min as shown in Table 6 and Figure 10. Hence, this optimized flow rate was used for all the studies conducted with GC-ECD. All the chromatographic data shown in the Results and Discussion were done in duplicate and the difference in the results of two runs was very small, being less than 10% in all the studies.

II. Studies of linear range

The linear range of a detector response is the range in which the detector response is directly proportional to concentrations of sample solutions or quantities of samples which are injected. The concept of linear range is particularly important from the viewpoint of detector calibration and quantitative determination on the basis of peak area or peak height. In order to achieve optimum quantitative analyses, it was necessary to study the linear range of newly studied compounds at specific column temperature and flow rates. The linearity ranges of permethrin and fenvalerate were investigated by GC-ECD on a 3% OV-275 column at the column temperatures of 175°C and 220°C, respectively.

Table 6. Height equivalent to a theoretical plate (cm) in relation to carrier flow rate (mL/min)*

Flow rate (mL/min)	retention time (cm)		N		HETP (cm)	
	cis	trans	cis	trans	cis	trans
27.3	3.4	4.2	826	849	0.184	0.179
30.0	3.1	3.9	956	968	0.159	0.157
33.3	2.9	3.7	916	916	0.166	0.166
40.0	2.6	3.3	776	783	0.196	0.194
46.2	2.4	3.0	697	710	0.218	0.214
47.6	2.3	3.0	676	694	0.225	0.219

* 1 µL injection of 7.5 µg/mL permethrin solution

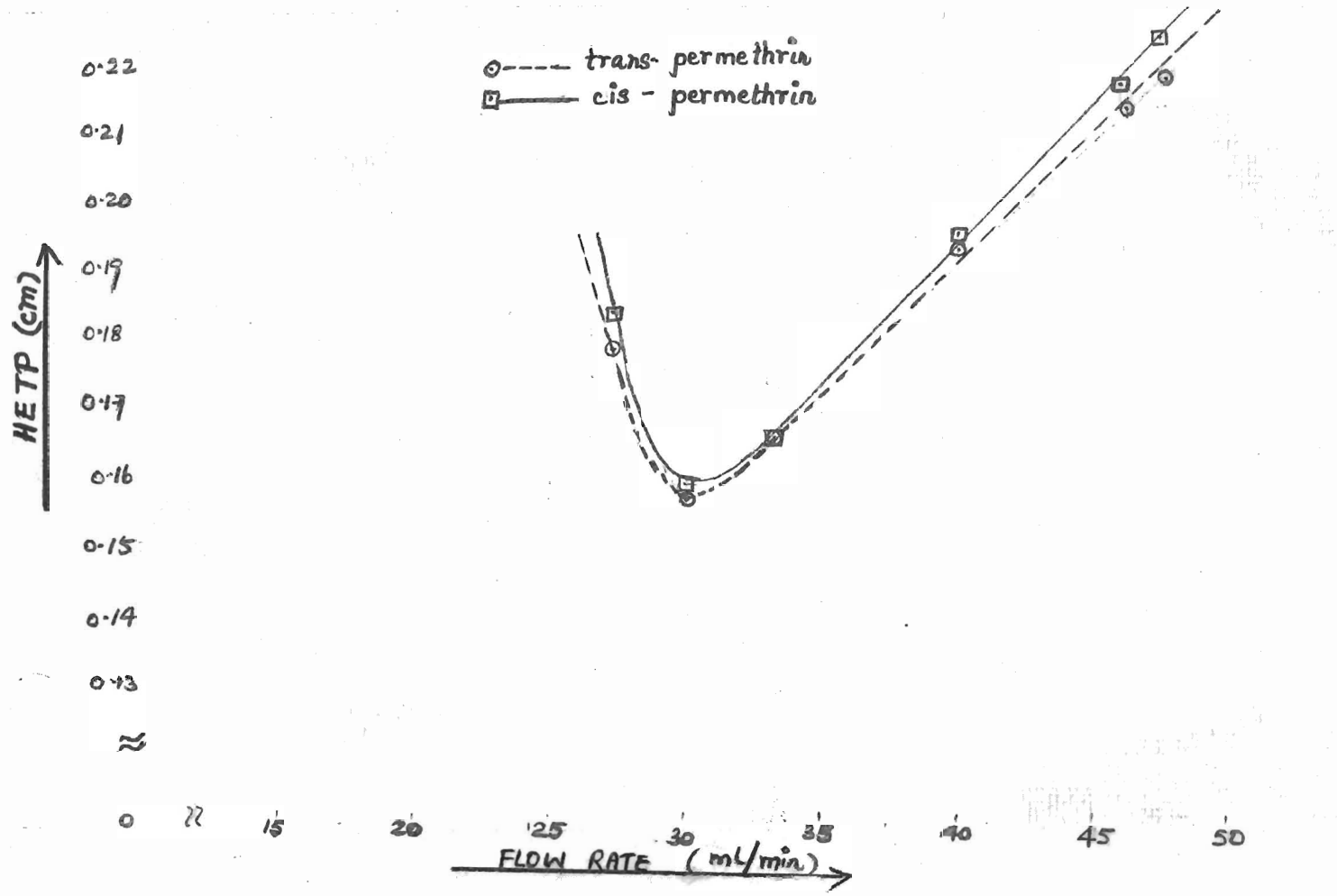


Figure 10. Height equivalent to a theoretical plate (cm) in relation to carrier flow rate (mL/min).

1. Linear ranges of cis- and trans-permethrin

The linear ranges of the detector response with the analytical standards of cis- and trans-permethrin were investigated at the concentration ranges from 1.0 µg/mL to 20.0 µg/mL in hexane as summarized in Tables 7 and 8, respectively. It is observed in Figures 11 and 12 that the response measured by peak heights of cis- and trans-permethrin was linear up to the concentration of 16.0 µg/mL and 20.0 µg/mL, respectively. This range of linearity is enough because the residue concentrations of cis- and trans-permethrin in leaf extracts at the time of injection were well within this linearity range.

Under the same conditions, the minimum detectable concentrations of cis- and trans-permethrin were determined. The minimum detectable concentration of a compound is the concentration which gives a peak height which is twice the average noise level of the chromatogram.[83] Since 1 µL each of 0.1 µg/mL cis- and trans-permethrin gave the same peak height of 7.0 mm at the detector scale, 8×10^{-10} AFS, with the average noise level of 1.0 mm, the minimum detectable amount was calculated as 0.03 ng.

2. Linear range of fenvalerate

When fenvalerate was gas chromatographed under identical conditions to those used for permethrin, its retention time was longer and it was eluted in a broader peak. Hence, the operating column temperature was increased to 220°C for the linearity studies with fenvalerate. From this study, it was noted that the separation of fenvalerate isomers was impossible under the experimental parameters and the column used. The

Table 7. Relationship between concentration of cis-permethrin (µg/mL) and response on GC-ECD measured by peak height (mm)

Sample concentration (µg/mL)	Detector scale (AFS)	Peak Height (mm)
1.0	8×10^{-9}	7.0
3.0	8×10^{-9}	19.0
10.0	8×10^{-9}	58.0
16.0	8×10^{-9}	100.0

Table 8. Relationship between concentration of trans-permethrin (µg/mL) and response on GC-ECD measured by peak height (mm)

Sample concentration (µg/mL)	Detector scale (AFS)	Peak Height (mm)
1.0	8×10^{-9}	7.0
3.0	8×10^{-9}	21.0
10.0	8×10^{-9}	64.0
20.0	8×10^{-9}	120.0

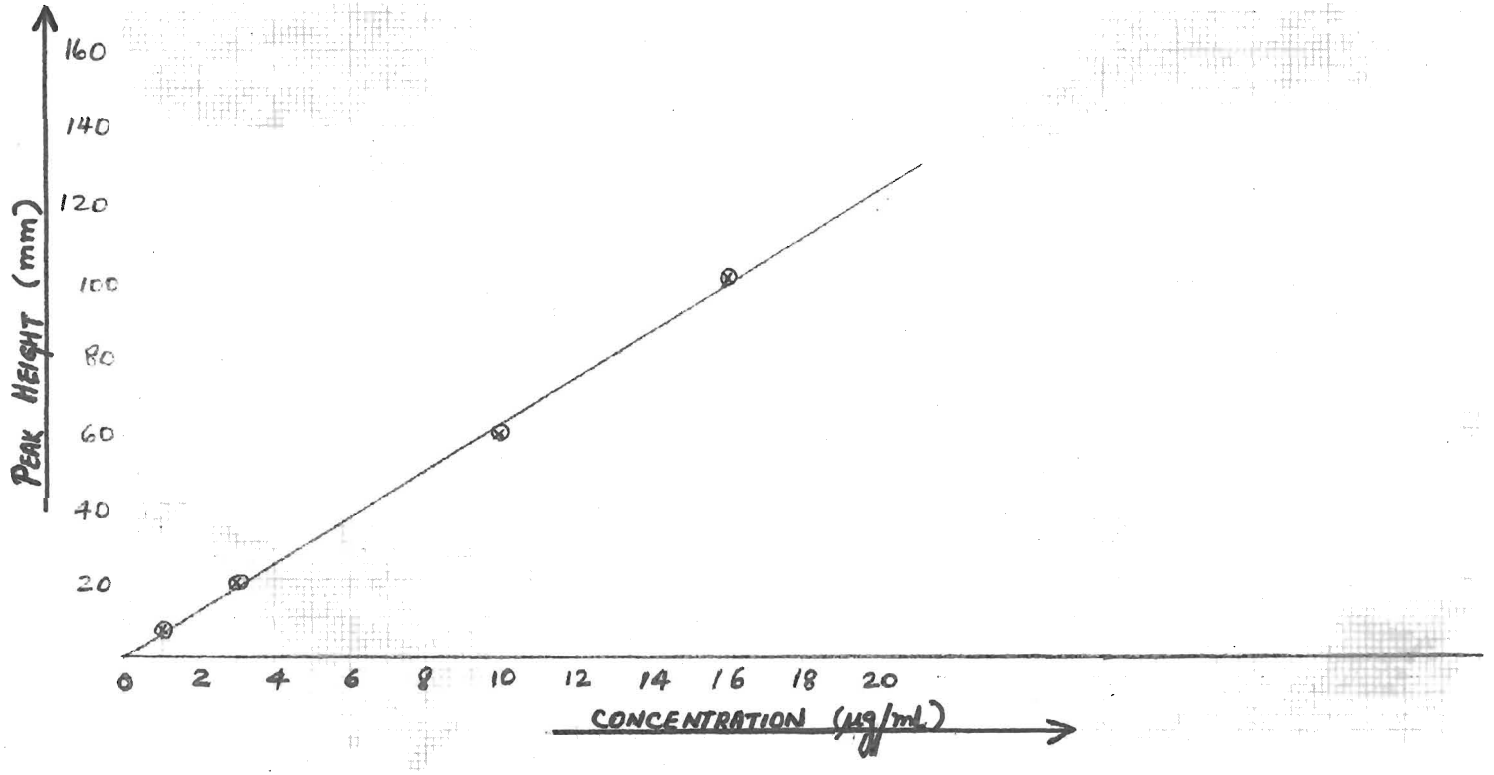


Figure 11. Response on GC-ECD, measured by peak height (mm) in relation to concentrations of cis-permethrin (µg/mL).

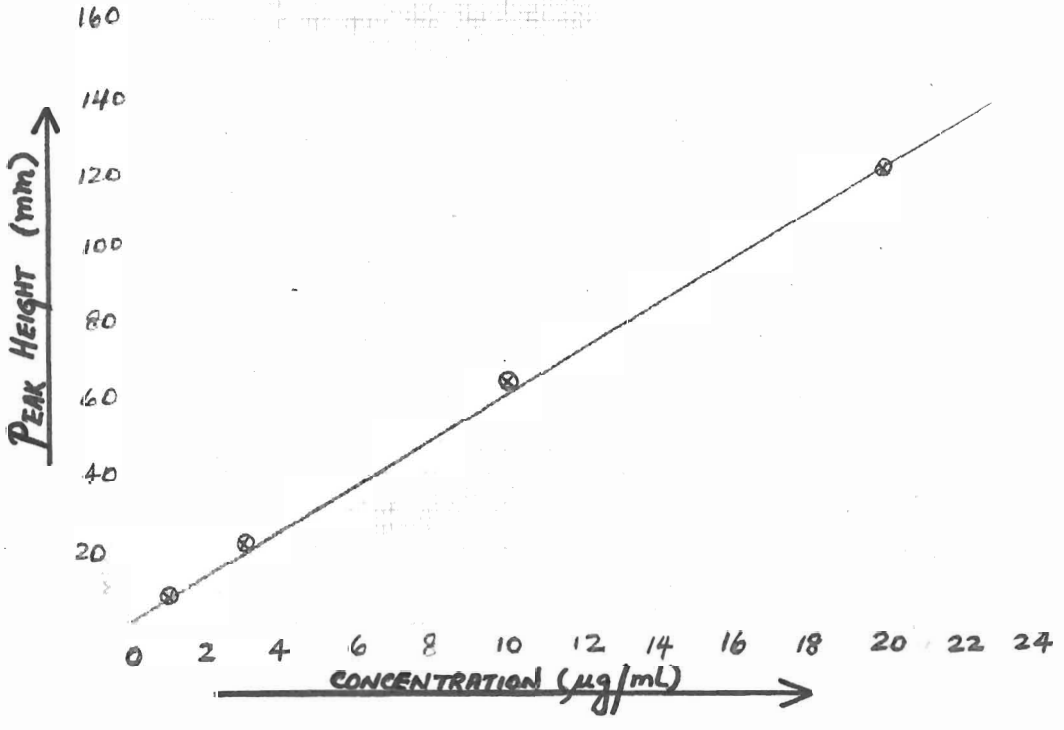


Figure 12. Response on GC-ECD, measured by peak height (mm) in relation to concentrations of trans-permethrin (µg/mL).

linear range of the standard fenvalerate solutions were examined with the following concentrations, 0.1 µg/mL, 0.4 µg/mL, 1.7 µg/mL and 4.3 µg/mL in hexane and the results are shown in Table 9, and also graphically plotted in Figure 13. It is observed from Figure 13 that the linearity, measured by peak heights, was good up to 4.3 µg/mL.

For the actual quantitative residue analyses, the concentration of fenvalerate used as external standard was less than 1.0 µg/mL, which is well within the linear range. It is illustrated in Table 9 that 1 µL of 0.1 µg/mL fenvalerate gave a peak with a 10 mm peak height, which showed the minimum detectable amount being 0.02 ng.

3. Linear range of 3-phenoxybenzyl alcohol

3-Phenoxybenzyl alcohol, PBalc (19), was found to be one of the major metabolites of permethrin in many studies conducted previously with a variety of soil and crop samples as discussed in the Introduction. Hence, the linear range of PBalc (19) was also studied by GC-FID with the standard solutions prepared in carbon disulphide at 1.1 µg/mL, 3.2 µg/mL 5.3 µg/mL and 10.6 µg/mL. During the studies, the column temperature was maintained at 180°C and gas flow rates were set as follows: nitrogen = 23 mL/min; hydrogen = 24 mL/min; and air = 429 mL/min. The observed results are summarized in Table 10 and plotted in Figure 14. As shown in Table 10, the 1 µL of 1.1 µg/mL PBalc (19) showed a peak with a 3.7 mm peak height; from this result the minimum detectable quantity was calculated as 0.6 ng.

Table 9. Relationship between concentration of fenvalerate (µg/mL) and response on GC-ECD measured by peak height (mm)

Sample concentration (µg/mL)	Detector scale (AFS)	Peak Height (mm)
0.1	8×10^{-10}	10.0
0.4	8×10^{-10}	42.0
1.7	$8 \times 10^{-10}/4 \times 10^{-9}$	154.0[a]
4.3	4×10^{-9}	376.0[a]

[a] Actual analyses were done at lower attentuation and calculated to meet the detector scale of 8×10^{-10} AFS.

Table 10. Relationship between concentration of 3-phenoxybenzyl alcohol (µg/mL) and response on GC-FID measured by peak height (mm)

Sample concentration (µg/mL)	Detector scale (AFS)	Peak Height (mm)
1.1	2 x 0.1	3.7
3.2	2 x 0.1	10.5
5.3	2 x 0.1	18.0
10.6	2 x 0.1	40.0

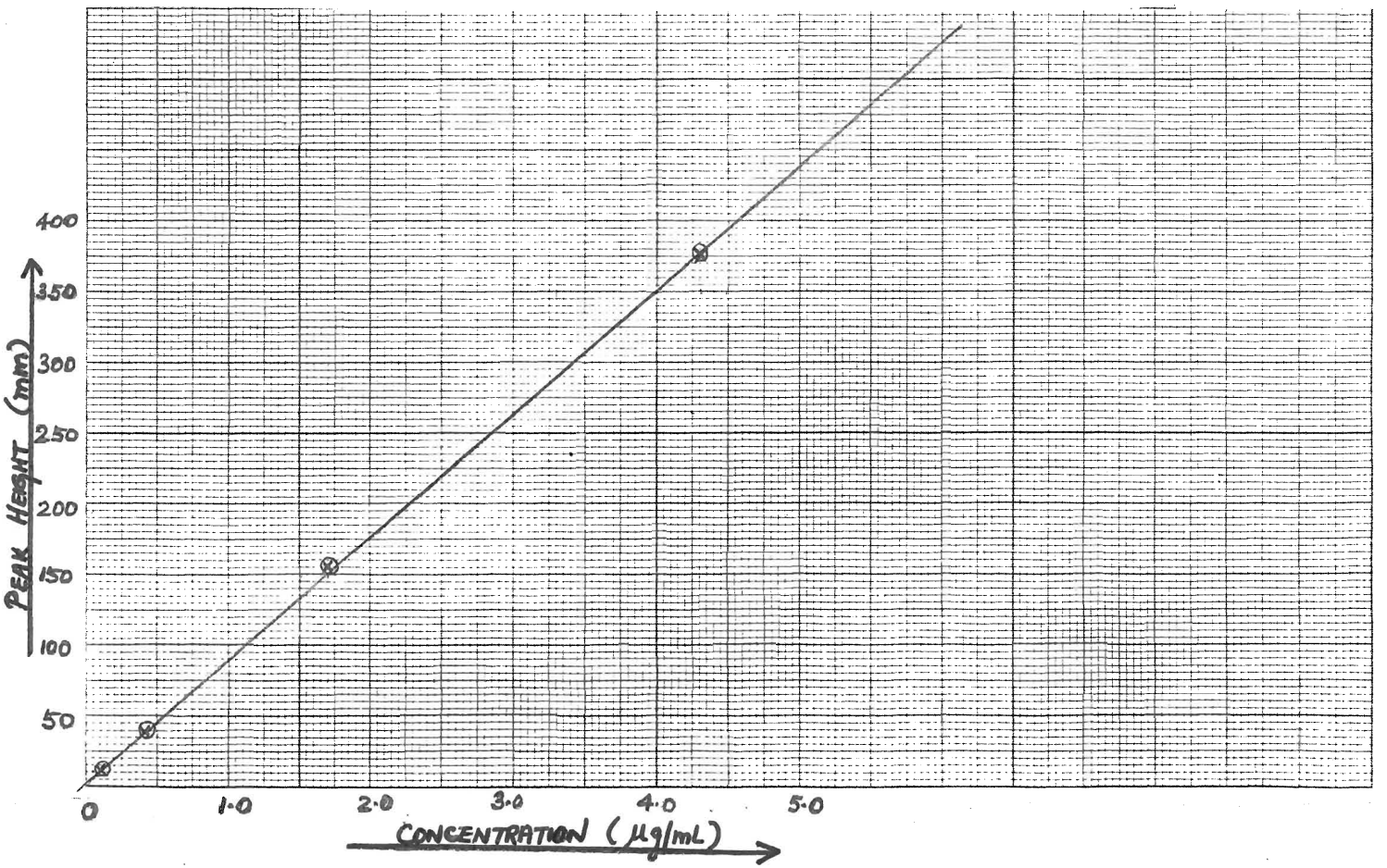


Figure 13. Response on GC-ECD, measured by peak height (mm) in relation to concentrations of fenvalerate (µg/mL).

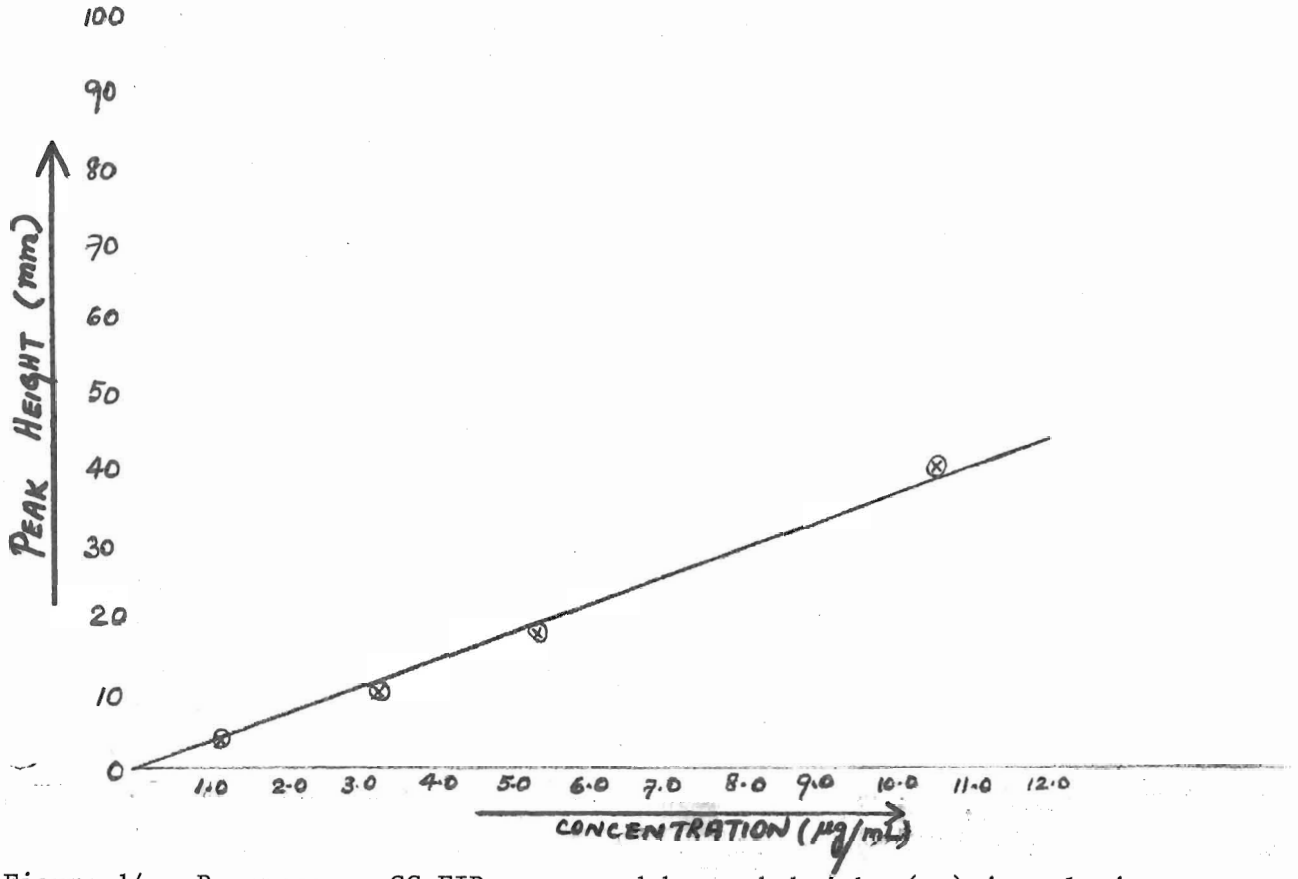


Figure 14. Response on GC-FID, measured by peak height (mm) in relation to concentrations of 3-phenoxybenzyl alcohol.

With actual leaf samples, PBalc (19) was extracted and partitioned into the hexane layer (fraction No. 1, Experimental section IV.3). It was thus necessary to transfer the solvent from hexane to carbon disulphide in order to avoid having a wide solvent front in the chromatogram. For this reason, the recovery study of PBalc (19) was done after having this solvent-transfer step. Before hexane was evaporated under a gentle stream of air, a drop of glycerol was added in order to prevent the loss of alcohol during this solvent transfer. Recoveries of PBalc (19) after this were 76% and 89% at 2.2 and 10.6 μg/mL levels, respectively.

III. Analyses of permethrin and fenvalerate

The concentrations of permethrin and fenvalerate in apple foliage were quantitatively analysed by GC-ECD. For the analyses, an aliquot of the hexane extract (fraction No. 1, Experimental section IV.3) was taken, in which the parent compound was expected to be contained, and diluted with hexane prior to determining the concentration. The control sample was also treated in the same manner as the sprayed samples in order to examine whether there are any co-extractives which would interfere with the determination of parent compounds. The quantitative analyses were based on peak height comparison with corresponding external standards of appropriate concentrations.

1. Quantitative determination of permethrin and fenvalerate in treated leaf samples by gas chromatography with electron capture detection

Since technical permethrin (25% wettable powder) was used in the actual field application, it was decided to use the same permethrin product as the external standard for the quantitative analyses. However, in order to follow this, it was necessary to determine the actual concentrations and ratio of cis- and trans-isomers in the technical product. This was accomplished by comparing the chromatographic peak heights of both isomers in the product with known concentrations of individual analytical grade cis- and trans- standards. The determination revealed that the actual ratio of cis- and trans-permethrin in the technical product was 39.9% cis-isomer and 60.1% trans-isomer. The order of elution of permethrin on a 3% OV-275 column was the cis-isomer first, and then followed by the trans-isomer with the retention times of 2.9 minutes and 3.8 minutes, respectively, at 220°C, as shown in Figure 15(C).

When the control sample in hexane extract, without any cleanup, was injected into GC-ECD, as shown in Figure 15(A), no co-extractive interfered with the determination of permethrin. The residue concentrations found in a sample taken on the day of application at one gram leaf equivalent were 13.5 µg for the cis-isomer and 19.2 µg for trans-isomer. The summary of the residue concentrations in apple foliage at various sprayed intervals is shown in Table 11 and the results are plotted in Figure 16.

It can be seen from Table 11 that the concentrations of cis- and trans-permethrin residues in 23 day sample were 4.0 µg/g and 7.9 µg/g of leaves which represented 29.6% and 41.1% of the initial deposits,

Figure 15. Gas chromatograms of apple leaf extracts in hexane and permethrin standard

(A) control (untreated) leaf
(B) 0 day permethrin treated sample
(C) permethrin standard solution

(1 and 2 are the peaks of cis- and trans-permethrin, respectively.)

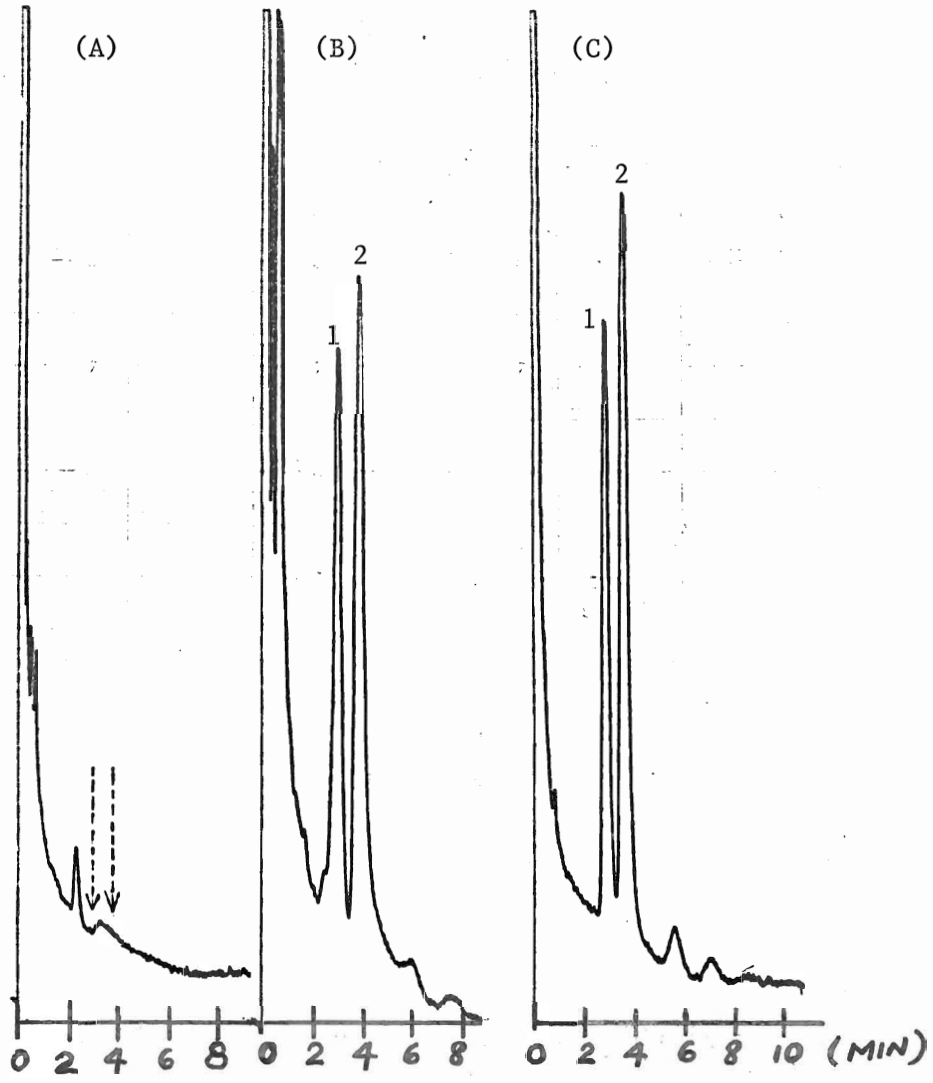


Table 11. Initial spray deposits and residues of permethrin at intervals (days) after spray application (μg/g of leaf)

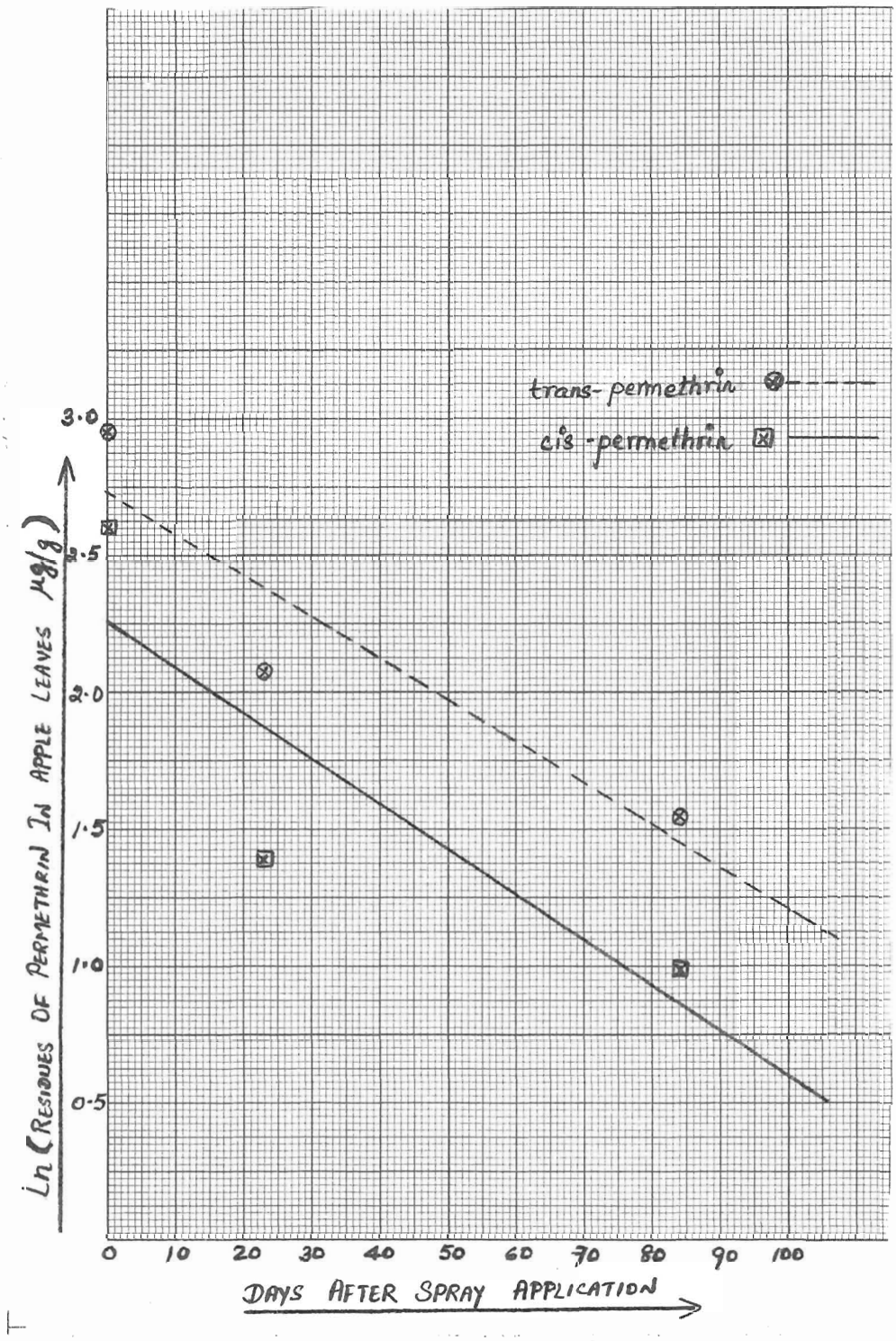
	0 day	23 days	84 days
	July 7, 1980	July 30, 1980	Sept. 29, 1980
cis-permethrin (μg/g of leaf)	13.5	4.0	2.7
after ln conversion	2.603	1.386	0.993
linear regression curve is	$y = a + bx = 2.251 - 0.0166x$		
correlation coefficient	$= -0.856$		
	0 day	23 days	84 days
trans-permethrin (μg/g of leaf)	19.2	7.9	4.7
after ln conversion	2.955	2.067	1.548
linear regression curve is	$y = 2.727 - 0.0151\ x$		
correlation coefficient	$= -0.919$		

Table 12. Initial spray deposits and residues of fenvalerate at intervals (days) after spray application (μg/g of leaf)

	0 day	23 days	84 days
fenvalerate (μg/g of leaf)	28.0	13.4	8.0
after ln conversion	3.332	2.595	2.079
linear regression curve is	$y = 3.154 - 0.0139\ x$		
correlation coefficient	$= -0.937$		

Note: The half life (τ) of cis and trans-permethrin, and fenvalerate was calculated from the following: $\tau = \frac{\ln 2}{b}$

Figure 16. Dissipation of permethrin residues in leaves in relation to time (days) after spray application



respectively. For the 84-day sample, the corresponding residues were 2.7 μg/g and 4.7 μg/g which indicated 20.0% and 24.5% of the initial deposits remained in the treated samples, respectively. Based on the results, the observed half-lives of cis- and trans-permethrin in apple foliage were found to be 42 days and 46 days, respectively.

Quantitative determinations of fenvalerate residues were also made under identical conditions as permethrin. Since fenvalerate showed a single peak, the quantitative determination of the residue measures as total isomers instead of individual isomers. As shown in Figure 17(C) fenvalerate gave a peak with a retention time of 4.9 minutes and a 73.0 mm peak height. The control sample, as shown in Figure 17(A), did not show any interference in the region of interest where fenvalerate appeared. The results of fenvalerate residue determinations at various spray intervals are summarized in Table 12 and are graphically plotted in Figure 18.

As shown in Table 12, the residue concentration of 0 day application was 28.0 μg/g whereas the spray deposits in 23 day and 84 day samples were 13.4 μg/g and 8.0 μg/g which represented 47.9% and 28.6% of the initial despoits, respectively. From these results, the observed half-life of fenvalerate in apple foliage was calculated as 51 days.

2. Studies of permethrin and fenvalerate by mass spectrometry

The mass spectra of the individual analytical standards, cis- and trans-permethrin, and technical fenvalerate showed molecular ion peaks ($M^{+\cdot}$) at m/z values 390 and 419, respectively. However, the presence of chlorine atoms in the molecular and fragment ions was recognized by the

Figure 17. Gas chromatograms of apple leaf extracts in hexane and fenvalerate standard

(A) control (untreated) leaf
(B) 0 day fenvalerate treated sample
(C) fenvalerate standard solution

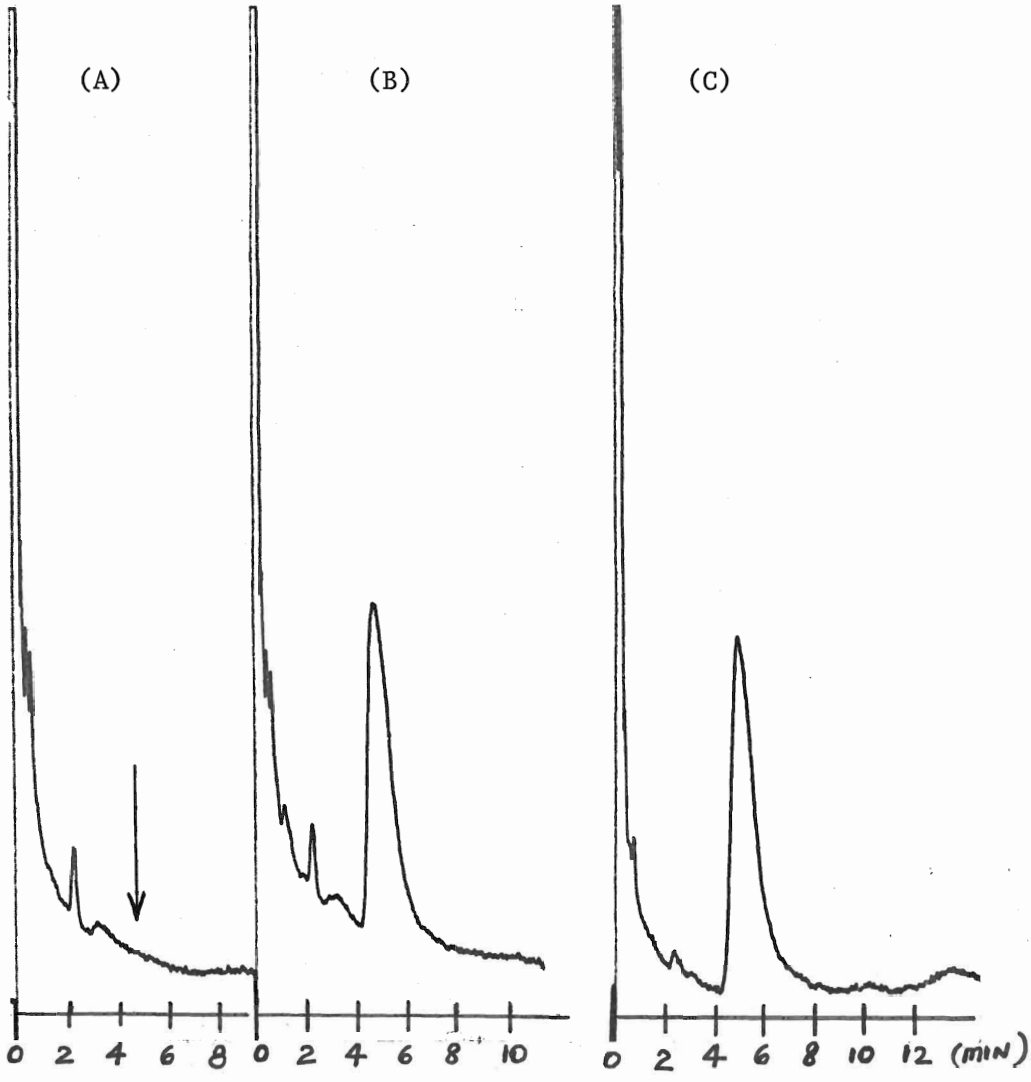
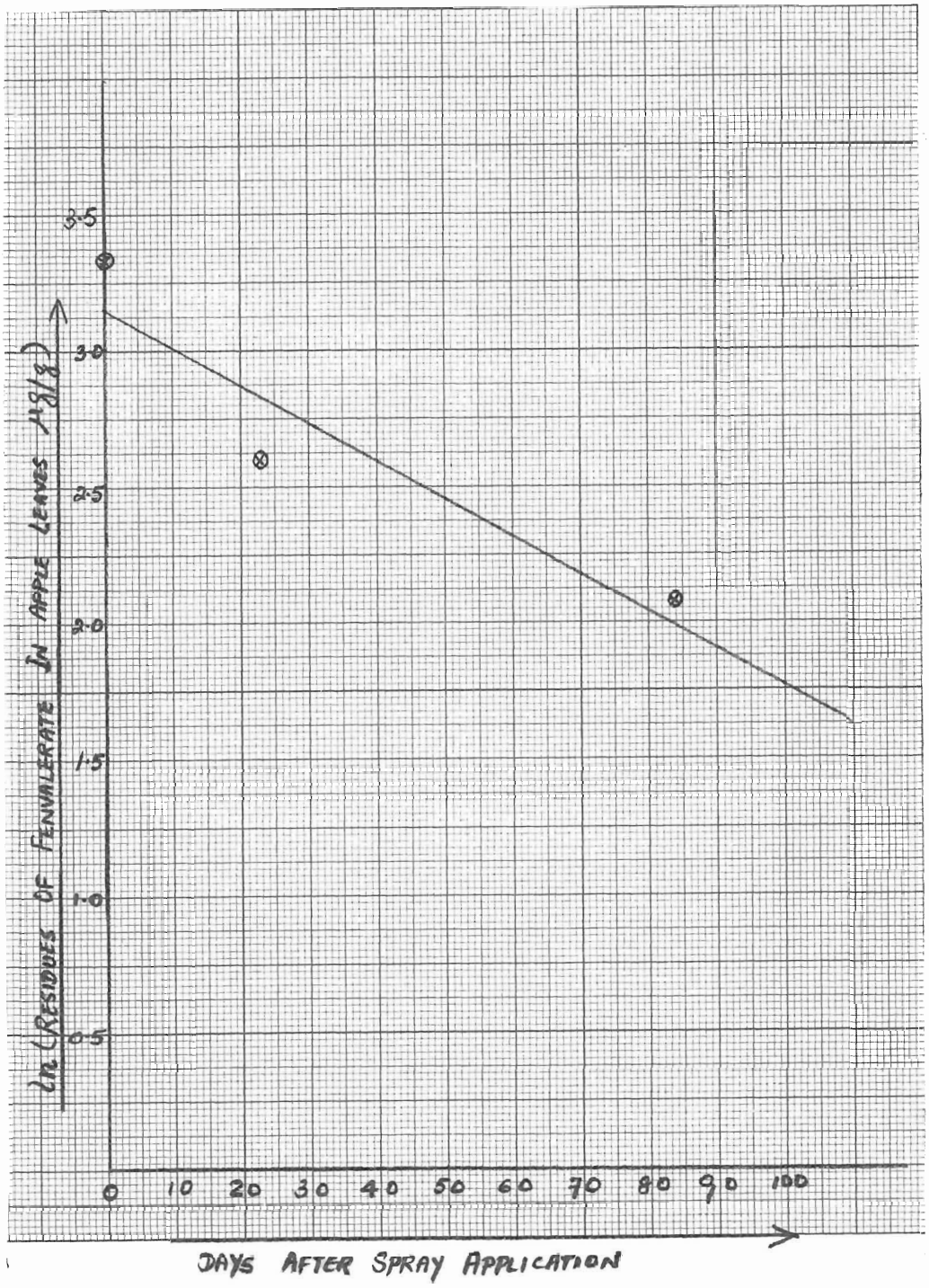


Figure 18. Dissipation of fenvalerate residues in leaves in relation to time (days) after spray application.



"isotopic clusters" in the spectra which are shown in Figure 19 (cis-permethrin), Figure 20 (trans-permethrin) and Figure 21 (fenvalerate). The peak intensities in Figures 19 and 20 were increased by a factor of 20 at m/z = 200 whereas the intensity increment was only three times at m/z = 250 in Figure 21.

The following m/z values are the major peaks of interest of these compounds, and the intensity ratios, normalized to the base peak, are given in parentheses after each m/z value. cis-permethrin (Figure 19) shows m/z 394 (0.2), 392 (1.0), 390 $M^{+\cdot}$ (1.5), 183 (100.0), 165 (52.2), 163 (60.9), 127 (17.3), 91 (25.1), 77 (83.8), 65 (10.7) and 51 (20.3). trans-permethrin (Figure 20) shows m/z 394 (0.4), 392 (2.5), 390 $M^{+\cdot}$ (3.8), 183 (100.0), 165 (75.8), 163 (74.6), 127 (26.1), 91 (34.9), 77 (42.5), 65 (12.0) and 51 (18.5). It should be noted from the above results that the spectra of the cis- and trans-isomers were very similar. Fenvalerate (Figure 21) shows the following fragment peaks, m/z 421 (5.4), 419 $M^{+\cdot}$ (17.8), 225 (29.2), 181 (27.3), 167 (62.6), 125 (60.1), 106 (50.5), 91 (100.0), 77 (26.9), 65 (8.1) and 51 (14.2).

3. Studies of permethrin by gas chromatography-mass spectrometry

Studies of permethrin were also conducted by GC-MS in order to determine the minimum detectable level as well as the characteristic of the peaks. The technical permethrin was analysed on a 3% OV-275 column with the temperature at 150°C for 2 minutes followed by increasing to 215°C at a rate of 10°C/minute. Under these conditions, 8 µL of 200 µg/mL permethrin gave peaks at retention times 7.1 minutes and

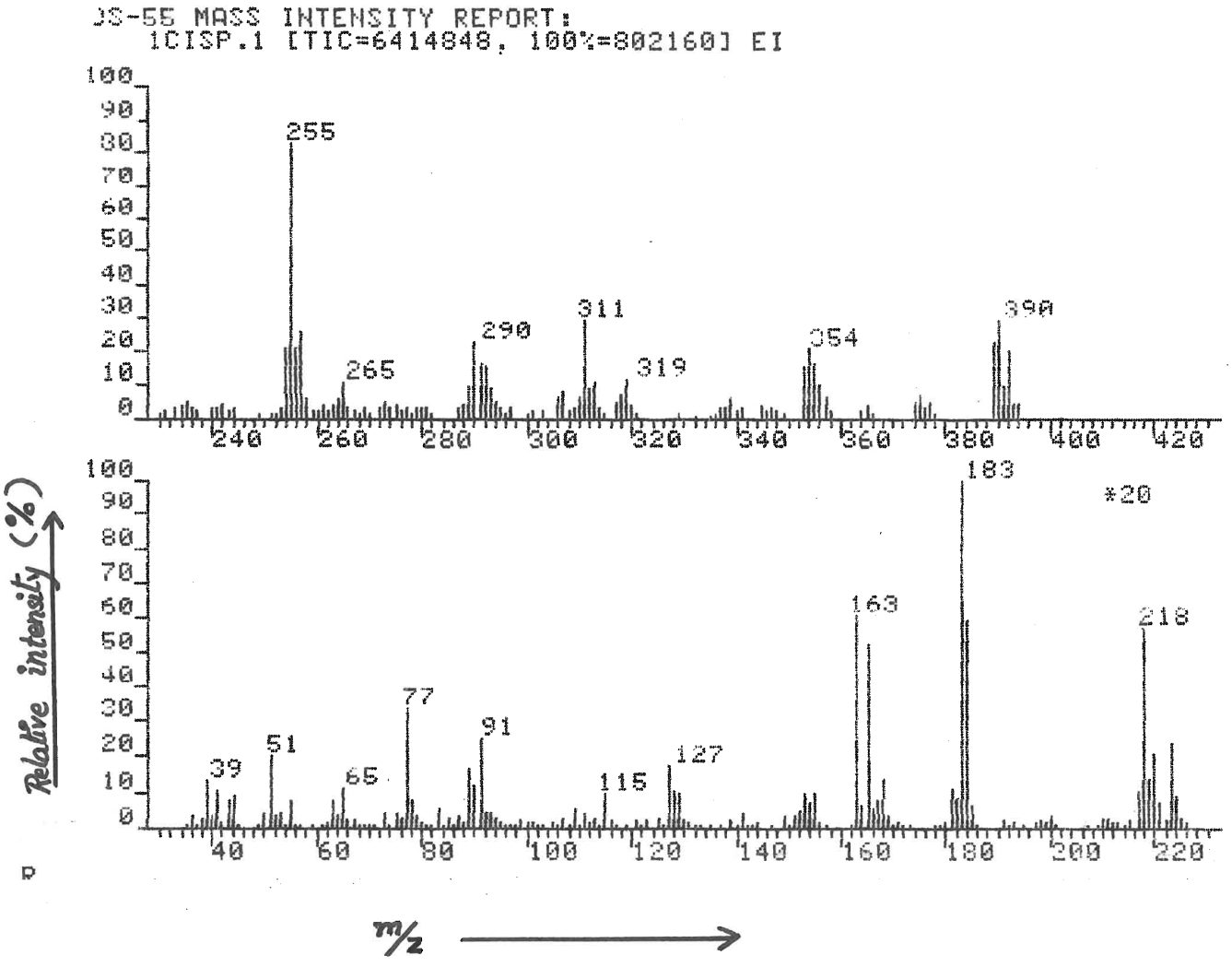


Figure 19. Mass spectrum of cis-permethrin.

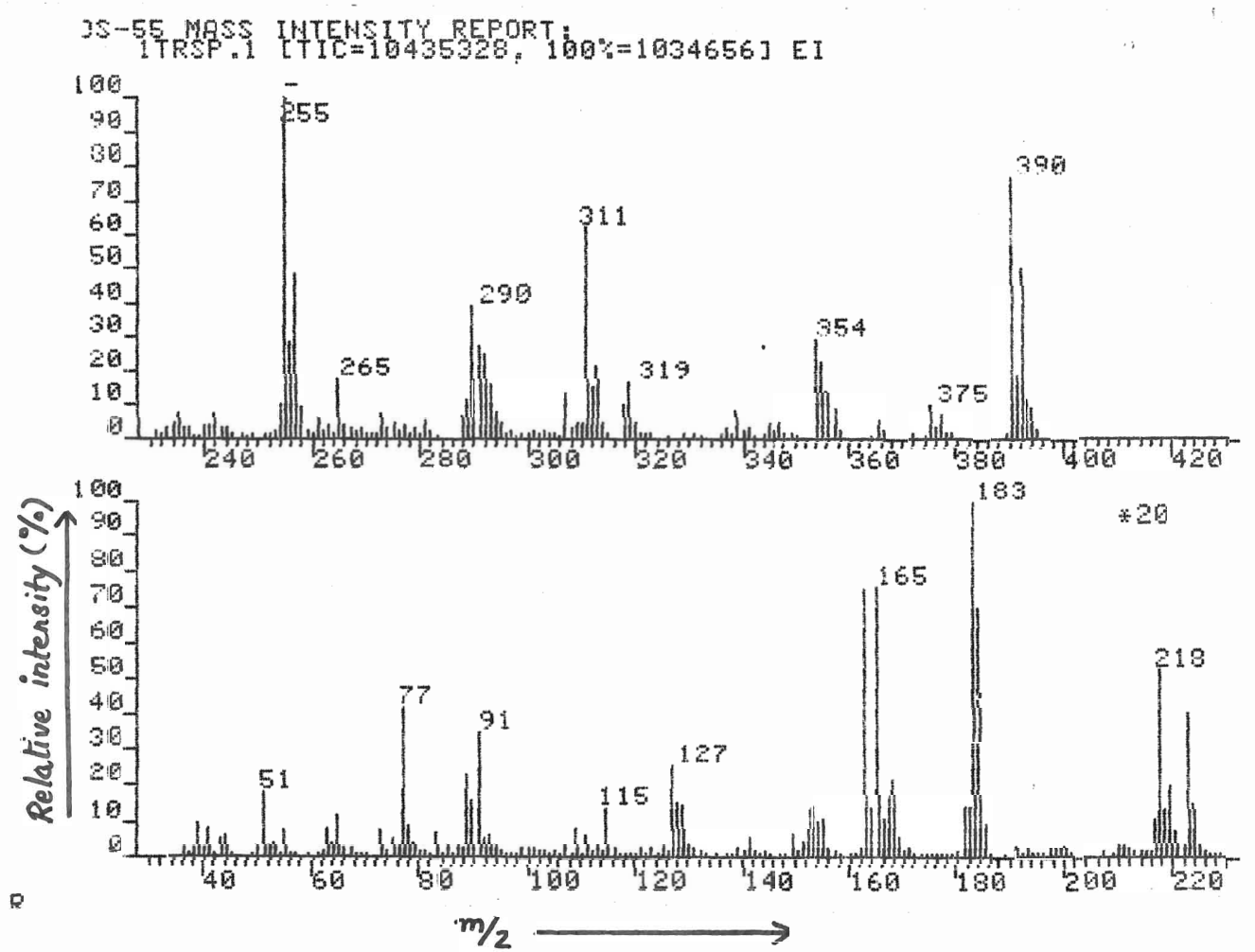


Figure 20. Mass spectrum of trans-permethrin.

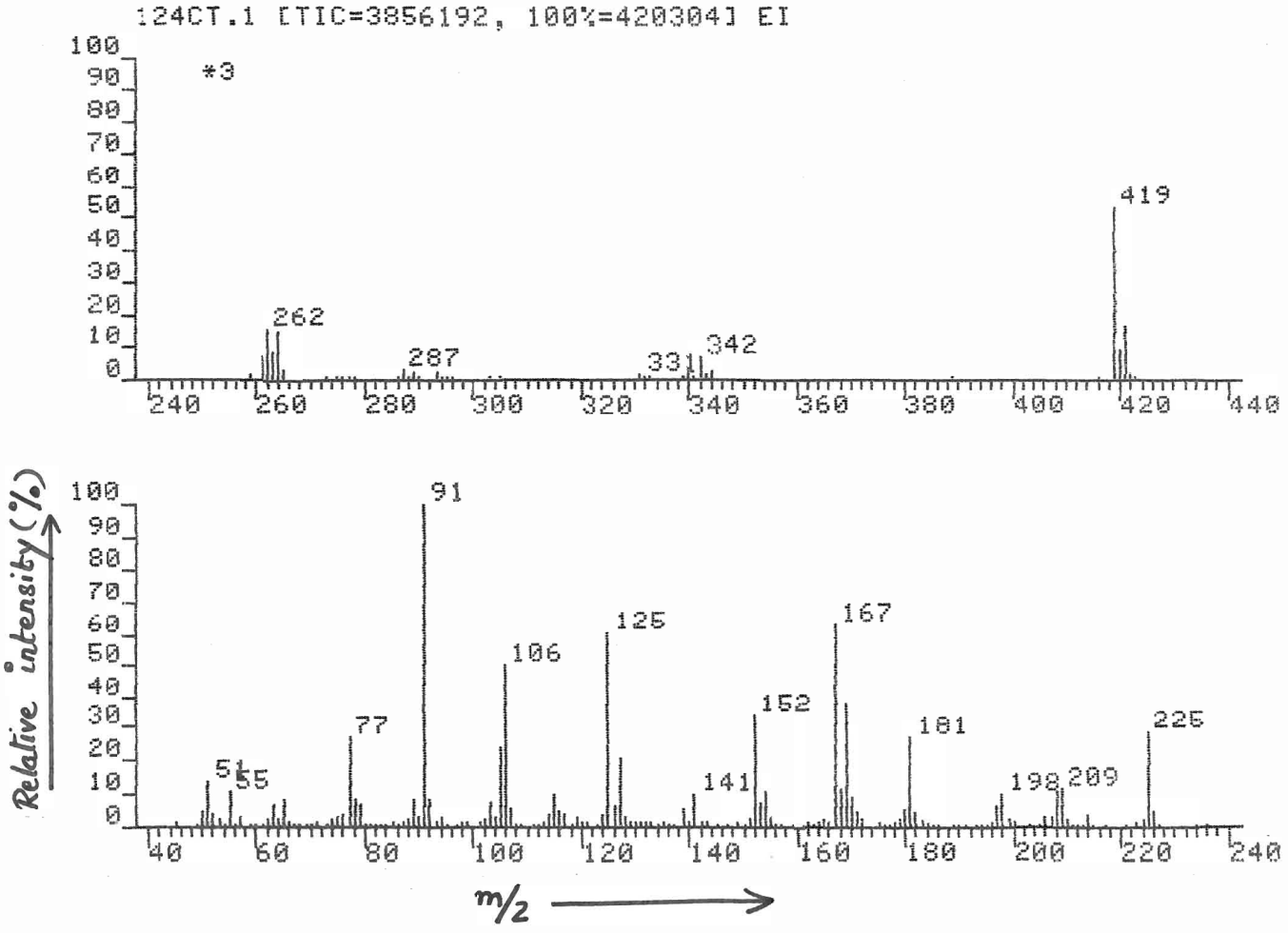


Figure 21. Mass spectrum of fenvalerate.

7.6 minutes with peak heights of 3.0 mm and 4.2 mm for cis- and trans-isomers, respectively. The mass spectra of the peaks were identical to those corresponding spectra mentioned in Section III.2. However, the study was repeated with a decreased sample size of 6 μL under the same experimental conditions; this time no signal was observed in the chromatogram. Hence, from the above observations and with an average noise level of 1.0 mm, the minimum detectable quantities were estimated to be 430 ng and 460 ng for cis- and trans-permethrin, respectively.

IV. Quantitative determination of alcohol constituents of permethrin and fenvalerate

Prior to the determination of the major alcohol constituents of permethrin and fenvalerate (i.e., PBalc (19) and PBald (38), respectively) in actual leaf extracts (fraction No. 1, Experimental section IV.3), the reference standards were analysed by GC-MS and GC-FID in order to obtain their fragmentation patterns and retention characteristics.

1. Studies of 3-phenoxybenzyl alcohol and 3-phenoxybenzaldehyde by gas chromatography-mass spectrometry

PBalc (19) was analysed on various columns and different operating conditions by GC-MS. The preliminary studies were performed with the 3% SE-30 and 5% QF-1 columns under the following operating conditions. The column temperature was initially set at 150°C for 5 min, then increased to 210°C at a rate of 15°C/min. In the study with a SE-30 column, 5 μL of 1000 μg/mL PBalc (19) showed a broad peak at a retention time

of 8.5 min with a 10 mm peak height whereas a 5% QF-1 column gave a peak at a retention time of 7.9 min with a 21 mm peak height. Since the latter column produced a better result, it was decided to repeat the analysis using a small sample size, 1 μL of 216 μg/mL PBalc (19), at an isothermal temperature of 190°C. At this time, the chromatogram showed a weak peak at 2.3 min with a 1.8 mm peak height which translated into a minimum detectable amount of 216 ng.

A further GC-MS analysis was made with a 3% OV-275 column and the following temperature programming: the temperature of the column was set at 175°C for 1 min then increased slowly at a rate of 5°C/min to 220°C. With this set up, 5 μL of 1000 μg/mL PBalc (19) showed a peak at 2.5 min with a 54 mm peak height. The result of GC-MS in Figure 22 indicates that PBalc (19) gives the molecular ion peak at m/z = 200 as the base peak. The major peaks in the mass spectrum are m/z 200 $M^{+\cdot}$ (100.0), 181 (18.1), 171 (13.3), 107 (21.9), 94 (37.0), 77 (51.0) and 51 (45.0). Based on the concentration and the peak height obtained, the minimum detectable amount was calculated as 185 ng. From all the above GC-MS studies, it was shown that a 3% OV-275 column gave the best result, hence it was decided to use the same type of column for further studies of PBalc (19) by GC-FID.

The analyses of alcohol constituent of fenvalerate, PBald (38), was also performed on a 3% OV-275 column. Since PBald (38) was obtained by base hydrolysis of fenvalerate, it was decided to study its purity and retention time relative to an analytical standard of PBalc (19). For the study, the mixed sample, which contained 2 μL of 4000 μg/mL PBald (38) and 1 μL of 6000 μg/mL PBalc (19), was used and analysed under the following

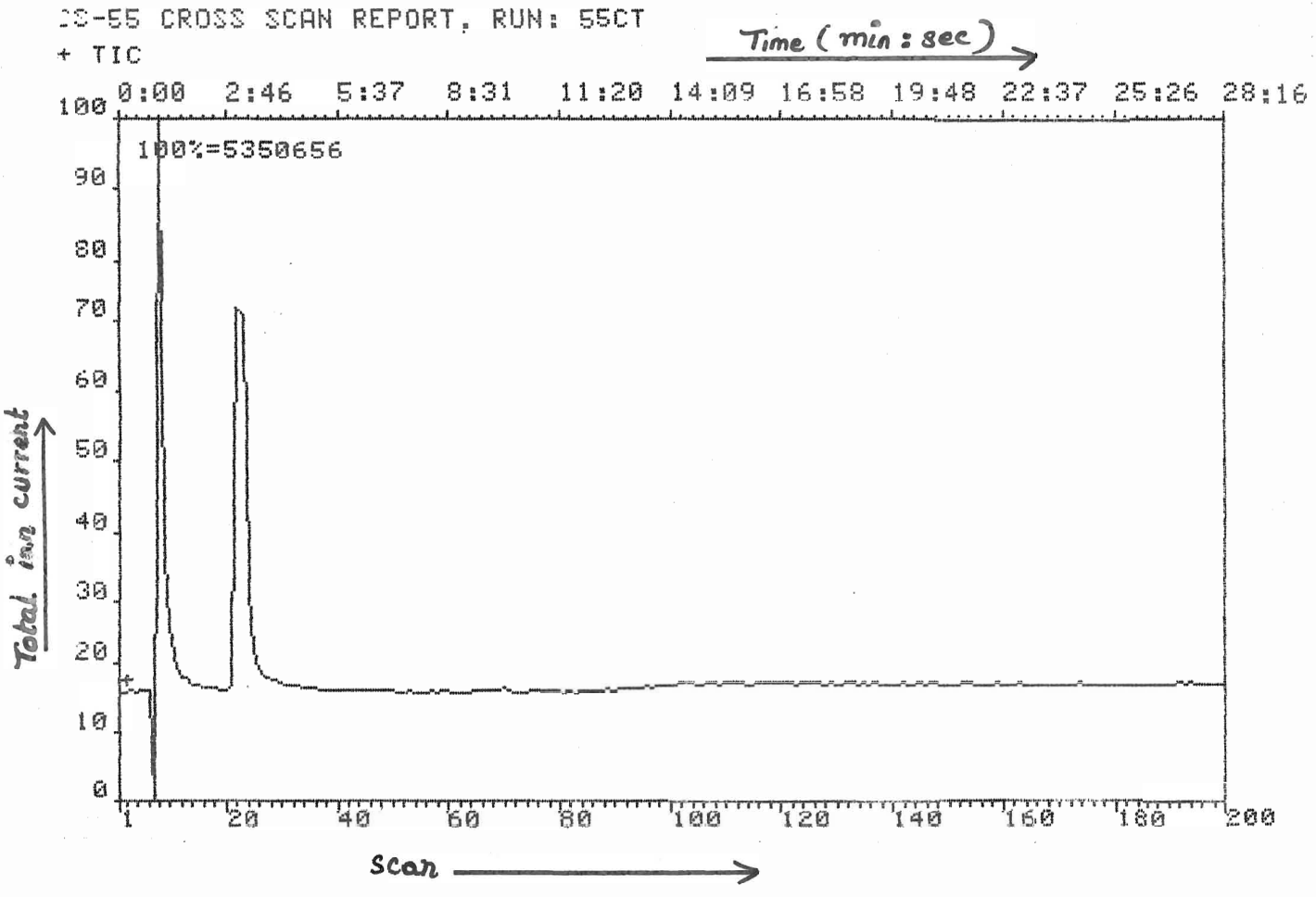


Figure 22(A). Gas chromatogram of 3-phenoxybenzyl alcohol.

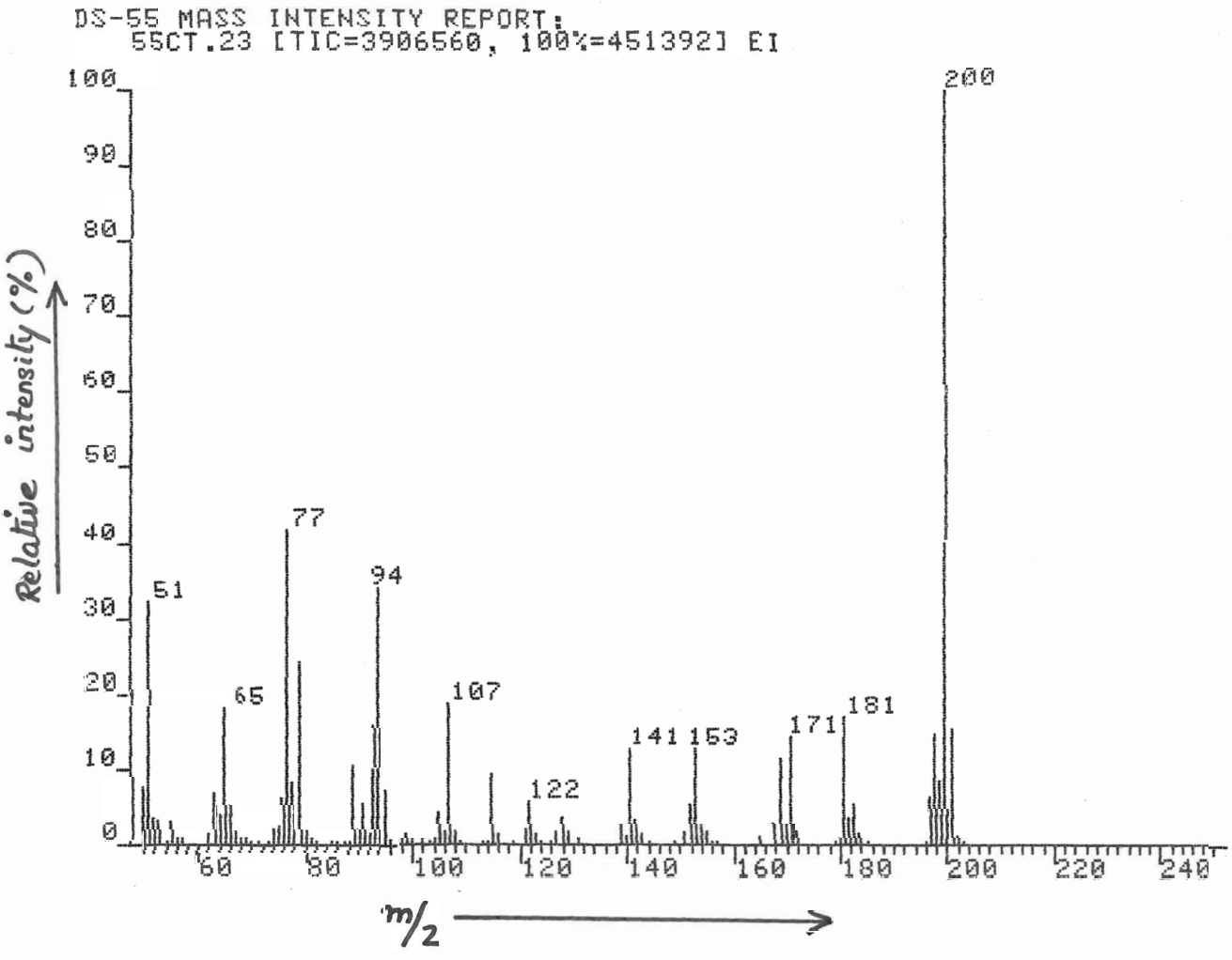


Figure 22(B) Mass spectrum of 3-phenoxybenzyl alcohol.

conditions: the column temperature was initially maintained at 30°C for 2 min, then increased to 210°C at a rate of 20°C/min. The resulting chromatogram and the corresponding mass spectra of the peaks are shown in Figure 23. The first peak in the chromatogram with a retention time of 9.8 min and a 68 mm peak height is due to PBald (38) and another peak at 11.2 min with a 20 mm peak height is referred to PBalc (19). The mass spectrum of PBald (38) shows the following major peaks: m/z 198 $M^{+\cdot}$ (88.3), 181 (30.0), 169 (83.7), 141 (97.2), 115 (54.4), 91 (36.7), 77 (81.6) and 51 (100.0). As the molecular structures of PBalc (19) and PBald (38) are similar, the mass spectra of these compounds resulted in similar fragmentation patterns.

However, the GC-MS run of PBald (38) itself was repeated under an isothermal condition at 170°C and the result is illustrated in Figure 24. In the chromatogram, Figure 24(A), 1 μL of 4000 μg/mL PBald (38) shows a single peak at 1.6 min with a 75 mm peak height and the mass spectrum obtained, Figure 24(B), is very similar to that of the previous one as shown in Figure 23(B). Hence, these results revealed that PBald (38) obtained was relatively pure and it could be used as the standard sample in further analyses. With the average noise level of 3 mm, the minimum detectable amount of PBald (38) was determined as 320 ng.

2. Study of silyl derivative of 3-phenoxybenzyl alcohol by gas chromatography-mass spectrometry

As the minimum detectable amounts of PBalc (19) observed from the above GC-MS studies were higher than that of the GC-FID study, it was felt

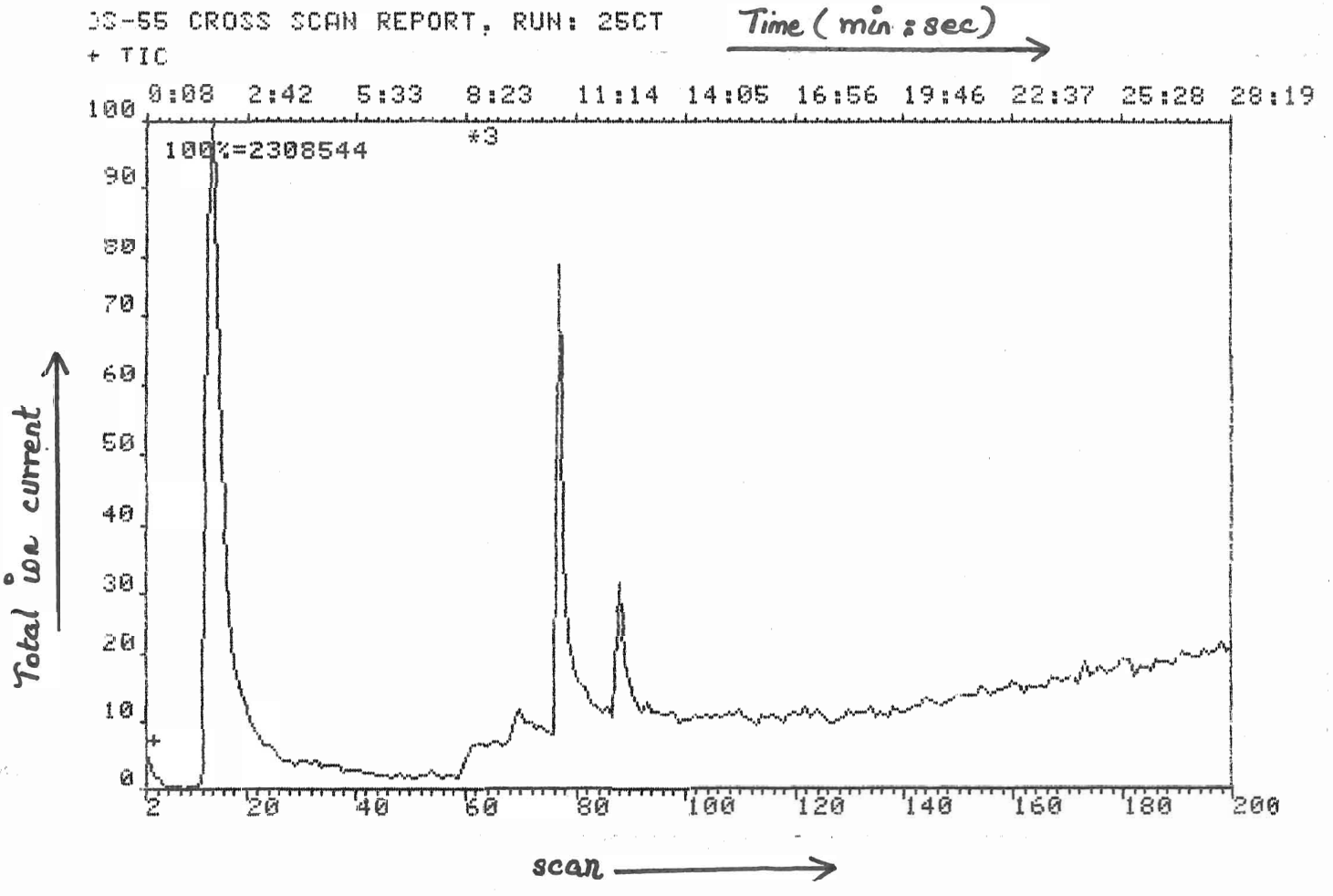


Figure 23(A). Gas chromatogram of mixture of 3-phenoxybenzaldehyde and 3-phenoxybenzyl alcohol.

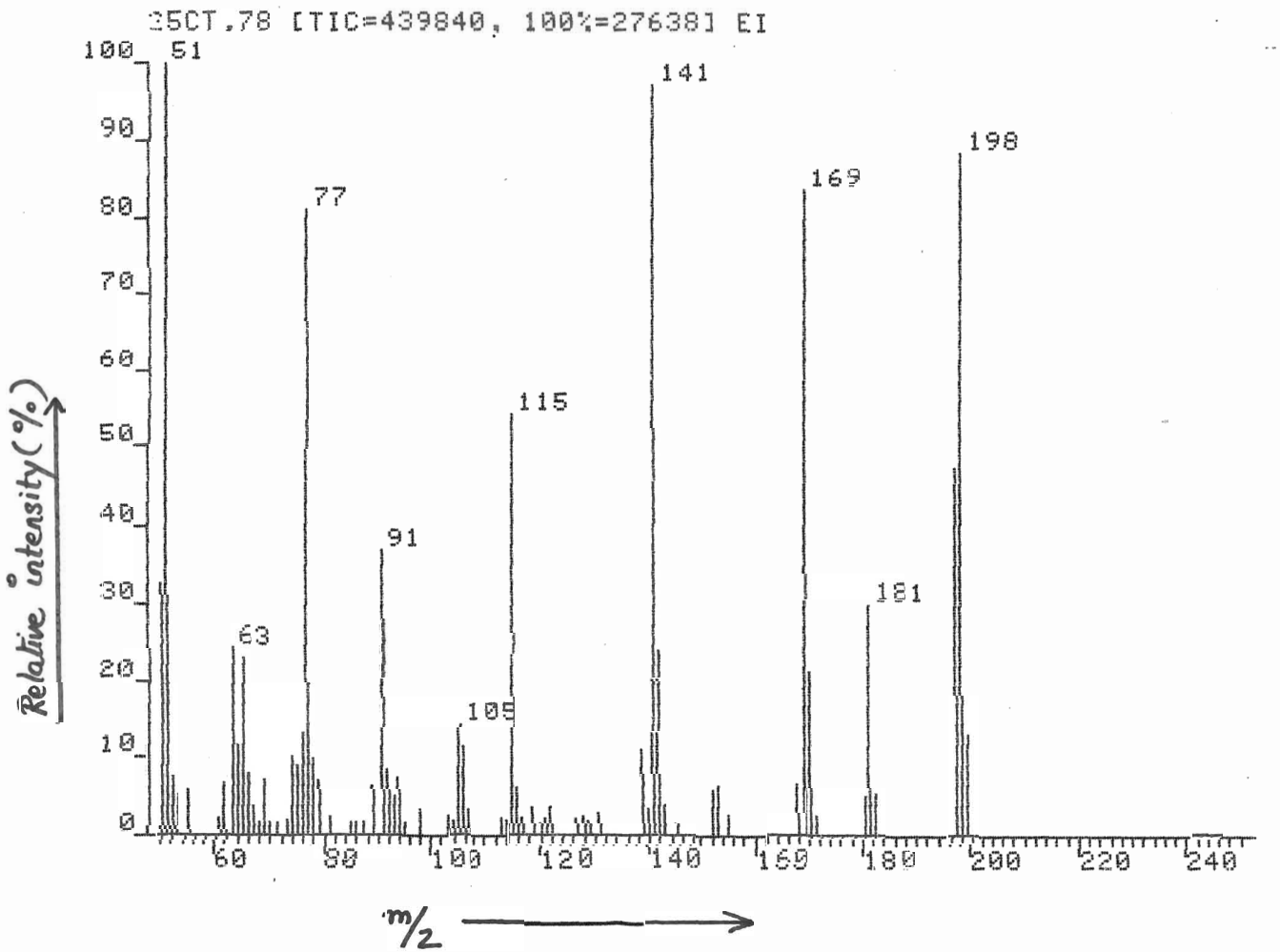


Figure 23(B) Mass spectrum of 3-phenoxybenzaldehyde.

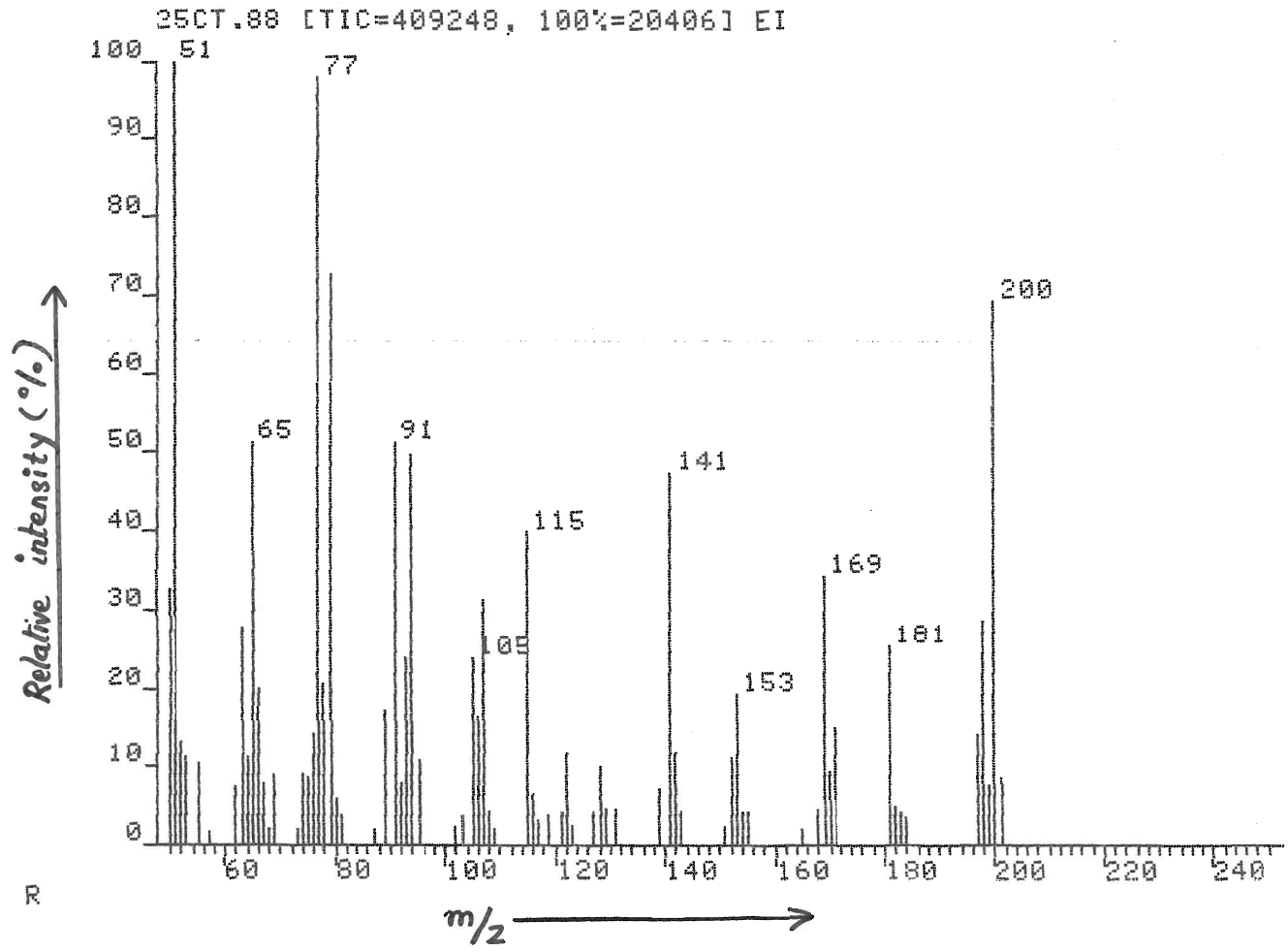


Figure 23(C) Mass spectrum of 3-phenoxybenzyl alcohol.

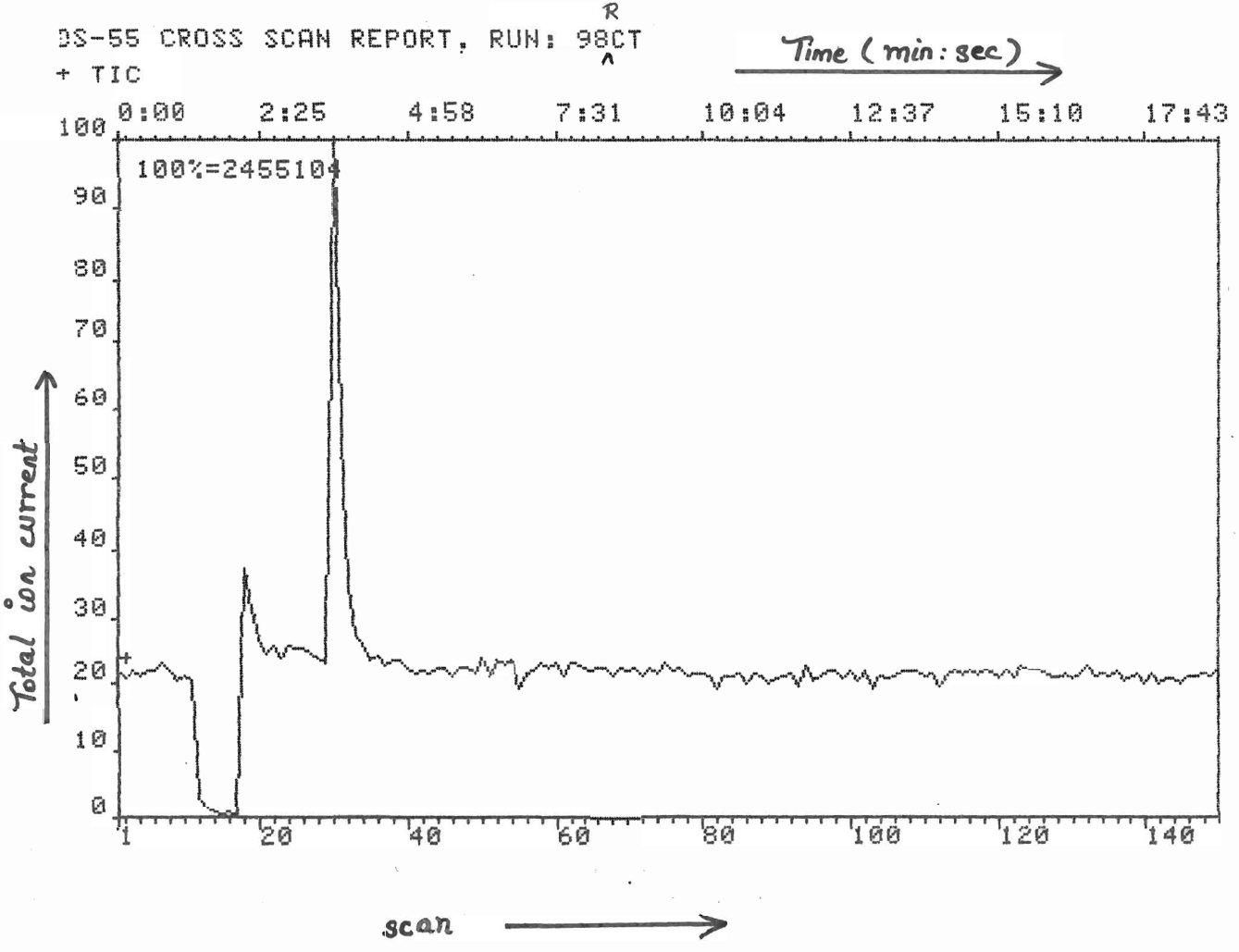


Figure 24(A) Gas chromatogram of 3-phenoxybenzaldehyde.

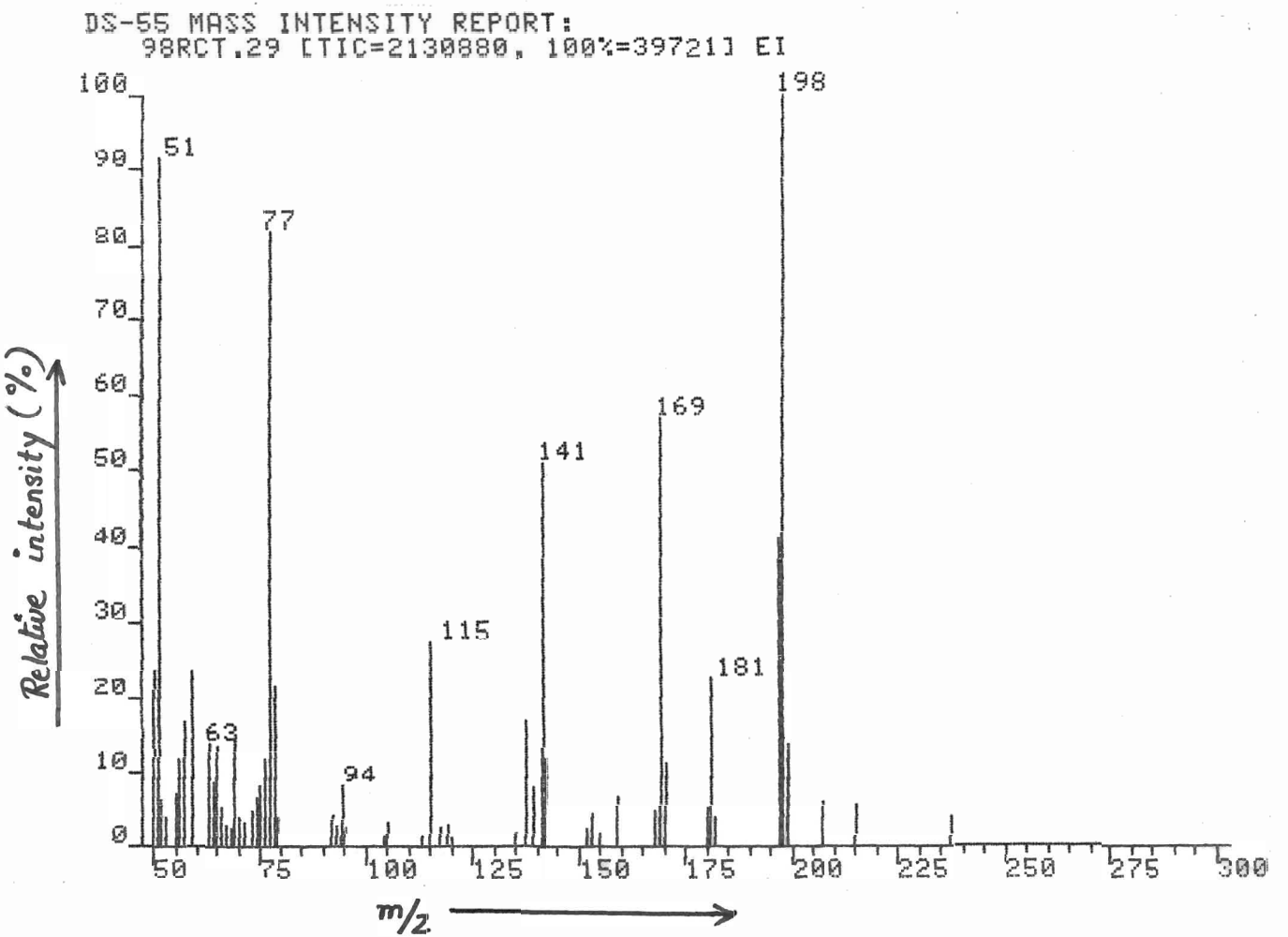


Figure 24(B) Mass spectrum of 3-phenoxybenzaldehyde.

that the derivatization of the alcohol to a more volatile product could allow operation at lower column temperatures as well as improving the detection sensitivity. Thus, PBalc (19) was converted to its silyl derivative by the reaction with t-butyldimethylchlorosilane/imidazole, as mentioned in Experimental section VI.

The silyl derivative was analysed on a 5% QF-1 column under an isothermal condition at 150°C. The silyl derivative of PBalc did not show the molecular ion peak at m/z 314, but it did give a peak at m/z 257 which was due to the loss of t-butyl group from the derivative; it agreed with the statement reported in the instruction sheet.[82] In the study, 1 μL of 2280 μg/mL of the silyl derivative gave a peak having a retention time of 6.7 min with a 25.2 mm peak height and an average noise level of 2 mm. The mass spectrum of the peak shows the following major peaks: m/z 257 (82.8), 227 (49.1), 183 (100.0), 115 (15.7) and 77 (25.7). From the above result, the minimum detectable amount of the silyl derivative of PBalc (19) was calculated as 362 ng. Since the sensitivity did not improve after the silyl derivatization, no further attempt was made.

From all the above information, it was realized that the quantitative analyses of the alcohol degradation products in actual leaf extracts would not be possible by GC-MS, due to a large quantity of sample required. However, as a result of these studies, the retention characteristics of PBalc (19) and PBald (38) in various columns under different operating temperatures and their electron impact mode mass spectra were obtained.

3. Studies of 3-phenoxybenzyl alcohol and 3-phenoxybenzaldehyde by gas chromatography with flame ionization detection

From the linearity study of PBalc (19) (Section II.3), it was observed that 1 μL of 1.1 μg/mL PBacl (19) gave a peak at a retention time of 3.3 min with a 3.7 mm peak height; from the result, the minimum detectable amount was calculated as 0.6 ng. With the same stationary phase, the minimum detectable quantity required by GC-MS was 185 ng; this result indicates that GC-FID is about 300 times more sensitive than GC-MS.

When PBald (38) was analysed by GC-FID under the column temperature at 180°C, as PBalc (19), 1 μL of 6.1 μg/mL PBald (38) gave a 119 mm peak at 0.8 min. It had already been shown by GC-MS in Section IV.1 that PBald (38) eluted earlier than PBalc (19), thus it was decided to decrease the column temperature from 180°C to 150°C in order to obtain a peak away from the solvent front. Under the new conditions, PBald (38) (with the same concentration and sample size), gave a peak at 1.7 min with a 29 mm peak height; from this result, the minimum detectable amount was calculated as 0.4 ng. From the GC-FID studies, it was realized that PBald (38) is approximately twice as sensitive as PBalc (19). The minimum detectable amounts obtained from the studies of GC-FID and GC-MS indicated that the former is about 800 times more sensitive for the analysis of PBald (38).

4. Quantitative determination of expected free alcohols of permethrin and fenvalerate in treated leaf extracts by gas chromatography with flame ionization detection

The expected alcohol constituent, PBalc (19) was sought in permethrin treated leaf extracts (23 and 84 days after application) by GC-FID. For the quantitative determinations, the control and treated leaf extracts in hexane (fraction No. 1, Experimental section IV.3) were transferred to carbon disulphide (0.2 g leaf/1 mL CS_2) and analysed at a column temperature of 180°C. Since the chromatogram of the control leaf extract showed an interference in the region of PBalc (19), attempts were made to remove the interference in the control sample by decreasing the operating temperature to 150°C, but the results were the same; i.e., there was still interference in the PBalc (19) region and the peak obtained with the treated sample was not any higher than that with the control sample.

Since the peak obtained with the treated sample was not any higher than that with the control sample, it was felt that the concentrations of the expected alcohol constituent in treated samples were probably not high enough to detect. Hence the control and treated leaf extracts were concentrated by 5 times (1 g leaf/1 mL CS_2) and run on GC again, but no improvement was observed. All the above attempts led to the conclusion that the concentration of expected PBalc (19) in the treated samples could either be lower than the minimum detectable limit, 0.6 ng, or converted to a conjugated alcohol product which was later extracted in the ether layer (fraction No. 3, Experimental section IV.3) or was further degraded to some other related products.

The quantitative determination of the expected PBald (38) was also studied, in the same manner as above, with the treated leaf extracts (23 and 84 days after application). In the studies, the control sample showed a significant interfering peak at the retention time of PBald (38) and the corresponding peaks with the treated samples were no larger than the control one. From these results, it was concluded that the expected PBald (38) in the treated leaf samples was not detectable, or its concentration was less than the minimum detectable amount of 0.4 ng if there was any.

5. Separation of expected free alcohol of permethrin from leaf extracts by column chromatography and its analysis by gas chromatography with flame ionization detection

Although GC-FID is relatively sensitive for analysing the reference standards of PBalc (19) and PBald (38), the quantitative determination of expected alcohol constituents in the treated leaf extract was difficult because there was a strong interference of the leaf coextractives. Hence, it was decided to cleanup the leaf coextractives by column chromatography (mentioned in Experimental section IV.4.a). Thirteen fractions were collected from each of the following samples: a control sample, a control sample plus known quantities of PBalc (19) and permethrin (8) (spiked control sample) and a treated sample. Then the collected fractions were concentrated individually prior to the determination of PBalc (19) on GC-FID and permethrin (8) on GC-ECD.

The reason for analysing the spiked control sample was to determine the fractions in which PBalc (19) and permethrin (8) would be contained and to perform their recovery studies. From the GC-ECD chromatograms, it was observed that permethrin (8) was eluted in the fractions Nos. 7 and 8 when 1.0% acetone/hexane (v/v) was passed through the column whereas PBalc (19) was collected in the fractions Nos. 10, 11, and 12 when 5.0, 8.0 and 12.0% acetone/hexane mixtures (v/v) were used. The study of total recovery of peremthrin (8) in the spiked control sample by GC-ECD showed 81% recovery. The total concentrations of cis- and trans-permethrin found in the treated sample (84 days after application) were 2.1 and 3.8 μg/g, respectively. As shown in Table 11, the concentrations of cis- and trans-permethrin in the same sample without cleanup procedure were 2.7 and 4.7 μg/g, respectively. The reduction may be due to the loss during the cleanup procedure; the percentage loss was found to be 22% and 19% for cis- and trans-permethrin, respectively.

The study of total recovery of PBalc (19) in the spiked control sample by GC-FID was given as 138%; this figure indicated that plant coextractives were not completely separated, and thus resulted in a high percentage of recovery. Similarly, quantitative determination of the collected fractions, where PBalc (19) was expected to be present in the 84 day treated sample, was impossible because of the strong interference from the coextractives. No further attempt was made to improve this cleanup procedure.

6. Studies of expected free alcohol of permethrin in leaf extracts by high performance liquid chromatography

Since the cleanup procedure by column chromatography required a great amount of time and yet produced unsatisfactory results, it was decided to try another cleanup method: high performance liquid chromatography (HPLC). The instrument used for the study was a Spectra-Physics SP-8000 under the control of a microprocessor.

For the preliminary studies, 25 µL of 6.0 µg/mL PBalc (19) was introduced on a reverse phase Partisil ODS column and studied under the following conditions: a mixed solvent, consisting of 60% acetonitrile/water, was isocratically used as a mobile phase at a flow rate of 2 mL/min. With these conditions, the chromatogram of PBalc (19) showed a peak at 2.9 min with a 41 mm peak height and an average noise level of 1 mm; which translated to the minimum detectable amount as 7.3 ng. The fractions, (fraction No. 1, Experimental section IV.3) where PBalc (19) was expected to be present in the treated samples (23 and 84 days after application), were analysed under the same conditions as above. The chromatogram of the control sample gave a peak at the same retention time as PBalc (19), with a 9.0 mm peak height, whereas the treated samples resulted in a small shoulder on the slope of this impurity peak. Changing the composition of mobile phase to 50% acetonitrile/water did not improve the situation.

It was felt that the standard size column was too small to handle a large quantity of impurity which interferes with the detection of PBalc (19), thus a semi-preparative Zorbax ODS column was used under the following condition: the composition of the mobile phase was 70%

acetonitrile/30% water. With this condition, 12 μg/mL of PBalc (19) gave a peak at 2.8 min with a 65 mm peak height and the number of theoretical plates was found to be 3920. Under the same conditions, the control sample showed an interference at 2.9 min with a 15 mm peak height, whereas the 23 day permethrin treated sample gave a shoulder on a peak of 10 mm height in this particular retention time, but no signal was observed with the 84 day treated sample. Then 6.0 μg/mL of PBalc (19) and the control were studied with different mobile phase compositions in order to separate the interference of the control sample from the PBalc (19) region of interest. With 65% and 50% of acetonitrile in acetonitrile/water mobile phase compositions, PBalc (19) revealed peaks at 2.9 min and 5.8 min with the peak heights of 24 mm and 11 mm respectively, and the interference peak from the control sample came out at essentially the same retention times. The decreasing of the acetonitrile composition in the mobile phase not only resulted in poorer peak shapes of PBalc (19), but also increased the intensity of the interference peak from the control sample.

As the above studies did not give the satisfactory results, the effort was extended to try a two-column HPLC method.[78] For the study, the semi-preparative Zorbax ODS column (used as the first column) was connected to a Brownlee labs RP-18 (as the second column) via a switch valve, through which the effluent from the first column was directed to the detector or to the second column. When the study was first conducted with a 70% acetonitrile/water mobile phase and a flow rate of 3 mL/min, PBalc (19) showed a peak at 5.0 min on the first column by itself. After the retention time of PBalc (19) on the first column was known, PBalc (19)

was injected again and collected on the second column. Then the collected fraction was further analysed on the second column. With the same procedure, the control and the 23 day permethrin treated samples were studied. With this method, the problem still remained unresolved; the control sample showed a peak in the PBalc (19) region with a higher peak area than the treated sample. Then it was decided to analyse the collected sample on the second column with different mobile phase compositions: 50% and 40% of acetonitrile in the acetonitrile/water system, but no improvement was obtained. Moreover, the determinations required more time to complete one cycle as the system needed to equilibrate with the starting mobile phase composition. Hence, no satisfactory results were obtained from this two-column method.

Further attempts were made with the two-column system by using different mobile phase compositions with methanol/water, but no improvement was observed.

Since the reverse phase column system could not give an efficient separation, it was decided to try a normal phase separation with a semi-preparative Zorbax CN column. The mobile phase used was a mixed solvent, consisting of 80% hexane and 20% methylene chloride, and the flow rate was maintained at 4 mL/min. Under these conditions, 8.7 μg/mL of PBalc (19) showed a peak at 3.5 min with a 5.0 mm peak height; the number of theoretical plates obtained with this sytem was excellent, being 7600. However, the leaf extract still showed a strong interference at the retention time of PBalc (19), no analysis was possible with the treated samples.

Although many attempts were made to determine the expected PBalc (19) in permethrin treated leaf extracts, no satisfactory result was obtained from the HPLC studies. Hence, no attempt was made to determine PBald (38), a major alcohol constituent expected to be present in fenvalerate treated samples.

The reason for being unable to analyse the expected alcohol degradation product of permethrin, PBalc (19), was mainly due to the interference from the leaf coextractives. However, if the quantities of the degradation products are large enough, the identifications are possible. The results that there was no or little positive sign of the presence of expected degradation product in the treated samples indicated that there was no degradation product present or the quantity of the product, if present, was insignificant. Even if the degradation of permethrin took place and the expected PBalc (19) was once produced, it might have recombined with some plant constituent, for example, with glucose, and existed as a conjugated compound.[57,59] If this was the case, the expected compound should be found in the fraction No. 3, Experimental section IV.3.

V. Studies of acid constituents of permethrin by mass spectrometry

Since cis- and trans-Cl_2CA (18) are the major degradation products of permethrin, it was decided to obtain their mass spectra for characterizing their fragmentation patterns. Based on these results, the identification of the expected acid degradation products in the treated samples could be determined. The mass spectra of analytical standards of cis- and trans-

Cl_2CA (18) are illustrated in Figures 25 and 26, respectively. As shown in these figures, both isomers gave similar fragmentation patthers and the molecular ion peaks at m/z 208. The major peaks found in the cis-isomer are m/z 210 (3.5), 208 $M^{+\cdot}$ (5.3), 175 (24.2), 173 (78.7), 165 (32.5), 163 (48.6), 129 (17.6), 127 (35.0), 111 (23.1), 109 (29.8), 91 (100.0), 77 (57.8) and 51 (25.3). Similarly, the following prominent peaks are found in the trans-isomer: m/z 210 (3.0), 208 $M^{+\cdot}$ (4.6), 175 (33.2), 173 (100.0), 165 (54.0), 163 (79.9), 129 (24.8), 127 (49.6), 111 (32.1), 109 (42.9), 91 (93.0), 77 (45.5) and 51 (34.1).

VI. Quantitative determination of esterified products of acid constituents of permethrin and fenvalerate

Since the acid constituents of permethrin, Cl_2CA (18), and fenvalerate, CPIA (31), are not suitable for ordinary gas chromatographic analyses, they were converted to the following three different esters: (1) methyl ester by the reaction with diazomethane (Experimental section V.1), (2) phenacyl ester by the reaction with α-bromoacetophenone in the presence of potassium fluoride (Experimental section V.2), and (3) trichloroethyl ester by the reaction with the mixture of trichloroethanol and trifluoroacetic anhydride (Experimental section V.3). The individual esters thus formed were analysed by either GC-ECD, GC-MS or HPLC.

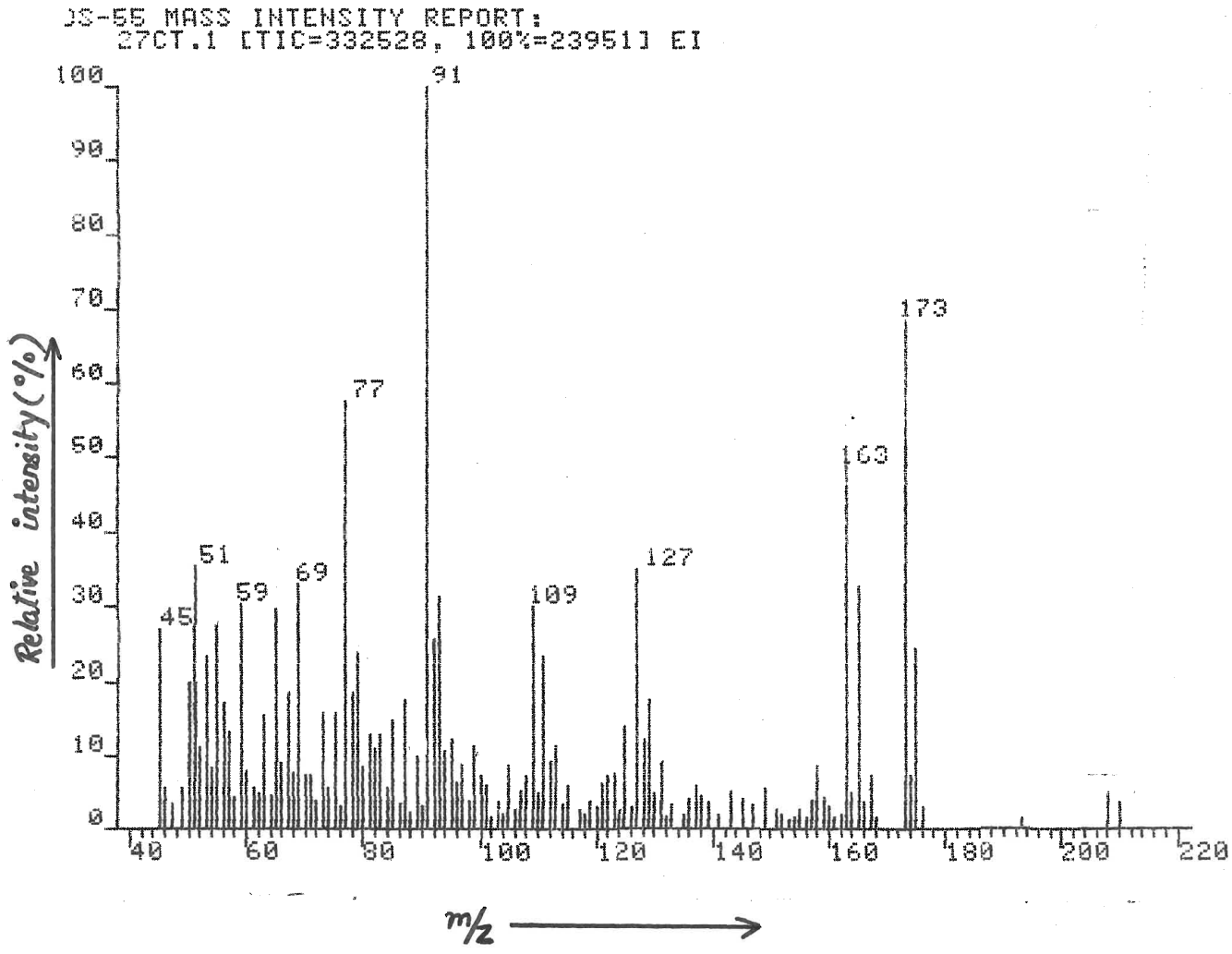


Figure 25. Mass spectrum of cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid.

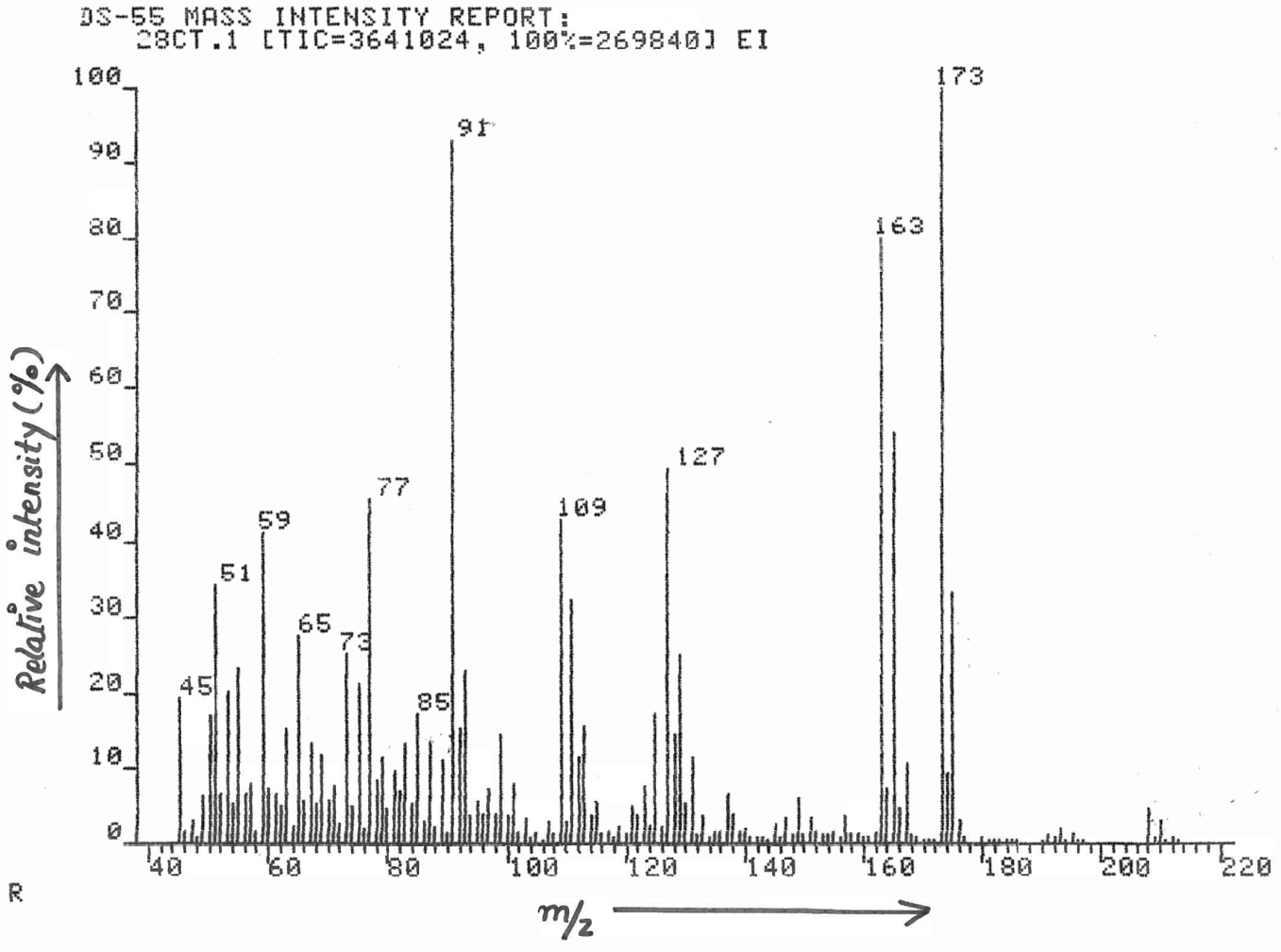


Figure 26. Mass spectrum of trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid.

1. Quantitative determination of methyl esters of acid constituents of permethrin and fenvalerate by gas chromatography-mass spectrometry and gas chromatography with electron capture detection

The methyl esters of analytical standards of cis- and trans-Cl_2CA (18) were initially identified by GC-MS on a 3% OV-275 column under the following operating conditions. The column temperature was maintained at 40°C for 1 min, then increased to 210°C at a rate of 20°C/min. The gas chromatogram of 5 µL of 7100 µg/mL trans-methyl ester showed a peak at 1.9 min, and its mass spectrum revealed the molecular ion peak at m/z = 222 as shown in Figures 27(A) and 27(B), respectively. The major peaks of the compound are observed as follows: m/z 187 (64.0), 165 (64.0), 163 (100.0), 129 (21.0), 127 (60.0) and 91 (87.2).

The same compound was analysed again at a lower concentration, 4 µL of 3600 µg/mL, and a slower temperature programming rate at 40°C for 1 min then increased to 180°C at a rate of 12°C/min. Under these conditions, the ester showed a peak at 4.9 min with a 21 mm peak height and a 1 mm average noise level, which indicated the minimum detectable amount as 1370 ng. Under the same conditions, 4 µL of 300 µg/mL cis-methyl ester revealed a peak at 4.3 min with a 1.5 mm peak height. The mass spectrum of the peak was identical to that of trans-methyl ester. Based on the observation obtained, the minimum detectable amount of cis-methyl ester was calculated as 1200 ng.

The methyl ester of CPIA (31) was also analysed by GC-MS on the same column under different operating conditions; the column temperature was initially maintained at 40°C for 4 min, then increased to 180°C with a

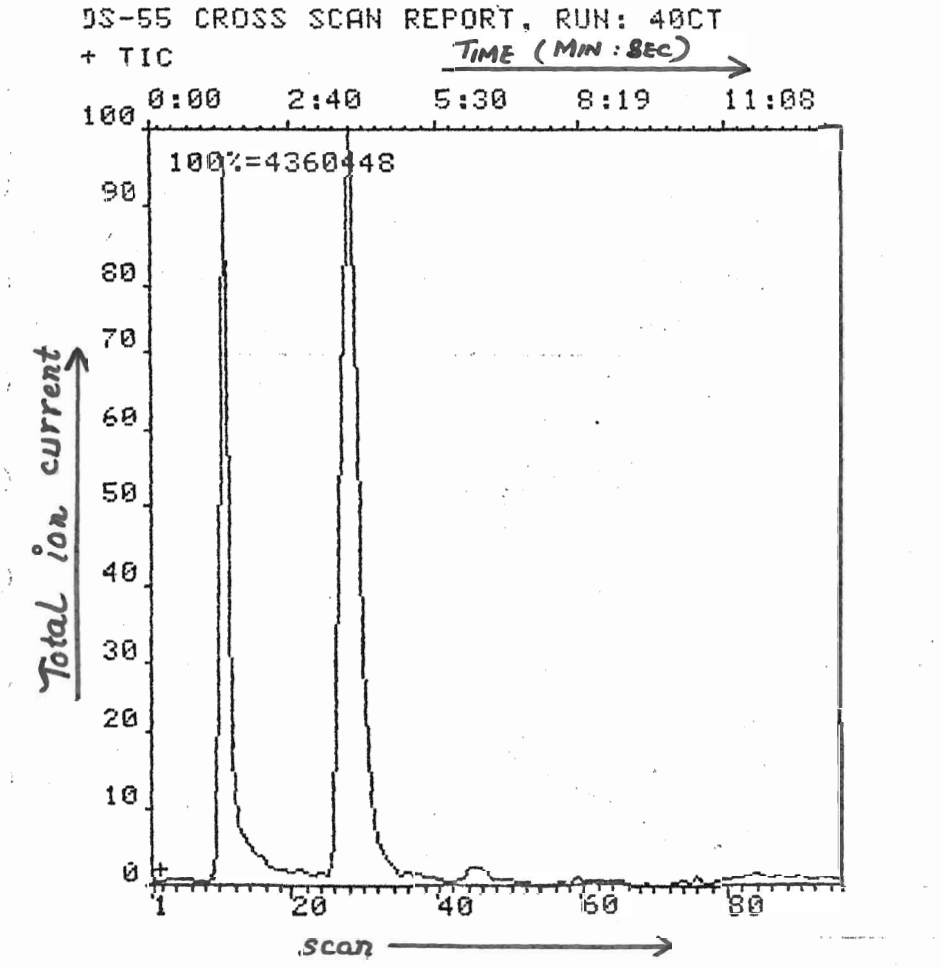


Figure 27(A) Gas chromatogram of methyl ester of trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid.

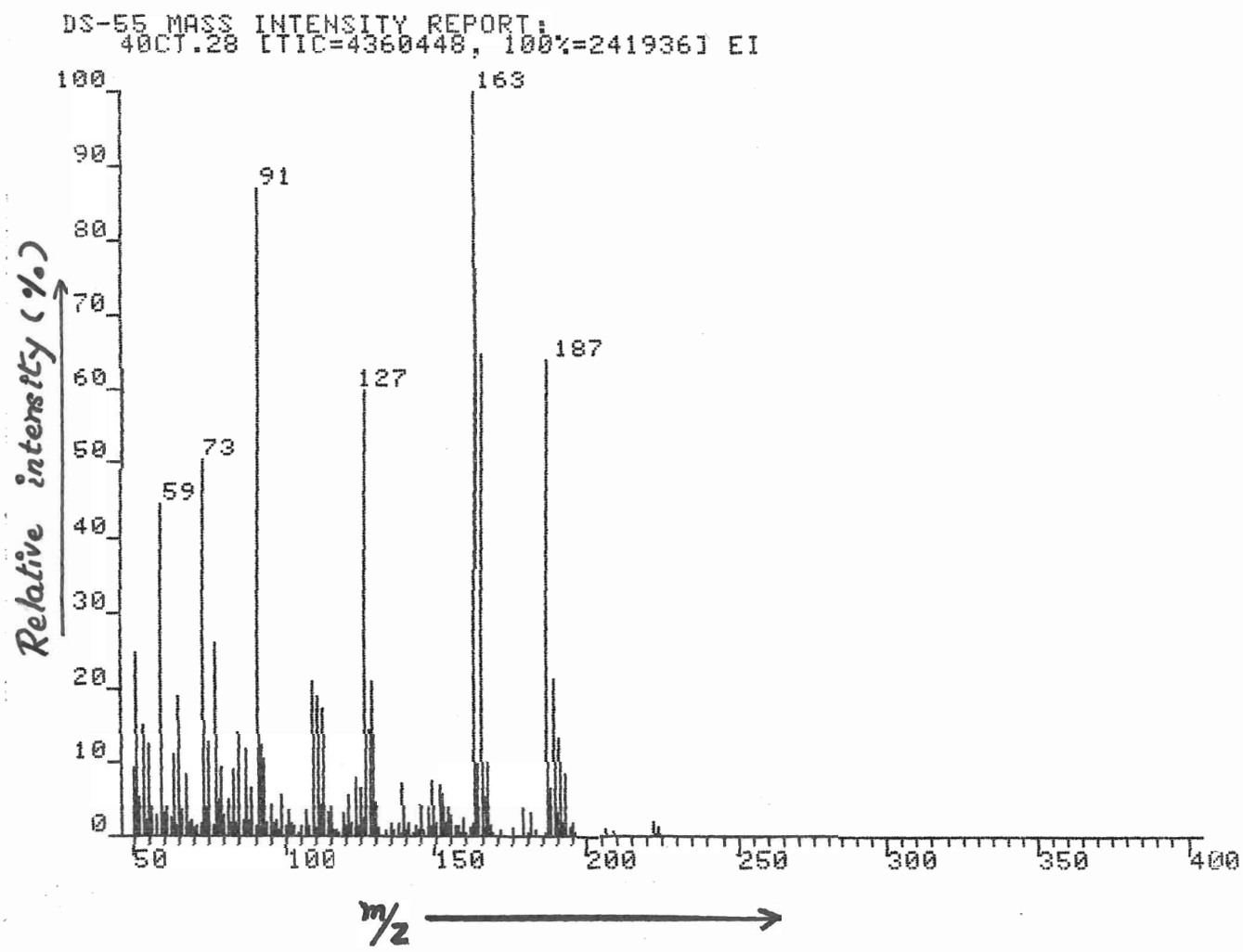


Figure 27(B) Mass spectrum of methyl ester of trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid.

rate of 12°C/min. Under these conditions, 1 μL of 3600 μg/mL of the ester gave a peak at 7.3 min with a 4.2 mm peak height, as shown in Figure 28(A). Since the ester contained one chlorine atom in the molecule, its mass spectrum shows one-chlorine isotopic cluster pattern at the molecular ion region. The prominent peaks observed in the mass spectrum, Figure 28(B), are as follows: m/z 228 (7.4), 226 $M^{+\cdot}$ (21.4), 186 (33.0), 184 (100.0), 167 (25.1), 152 (47.0), 125 (80.0) and 77 (24.1). Based on the above information, the minimum detectable amount of the methyl ester of CPIA (31) was found to be 1714 ng.

The methyl esters of cis- and trans-Cl_2CA (18) were also analysed by GC-ECD on a 3% OV-275 column under the following conditions. The temperature of the column was set at 80°C for 7 min and then increased to 200°C at a rate of approximately 10°C/min. Under the above conditions, 1 μL of 1660 μg/mL of the ester of the cis-isomer gave a peak at 2.6 min and a 123 mm peak height, whereas 1 μL of 1650 μg/mL of the ester of the trans-isomer showed a peak at 3.5 min and a 138 mm peak height. With the average noise level of 1.0 mm, the minimum detectable amounts of the esters of the cis- and trans-isomers were calculated as 27 ng and 24 ng, respectively.

From the results, it was obvious that the sensitivity of the methyl esters by the GC-ECD analyses was rather poor; there was no possibility of finding this level of residue in treated leaf samples. It was thus decided neither to analyse the methyl ester of CPIA (31) nor to derivatize the expected acid degradation products in the actual leaf extracts with diazomethane.

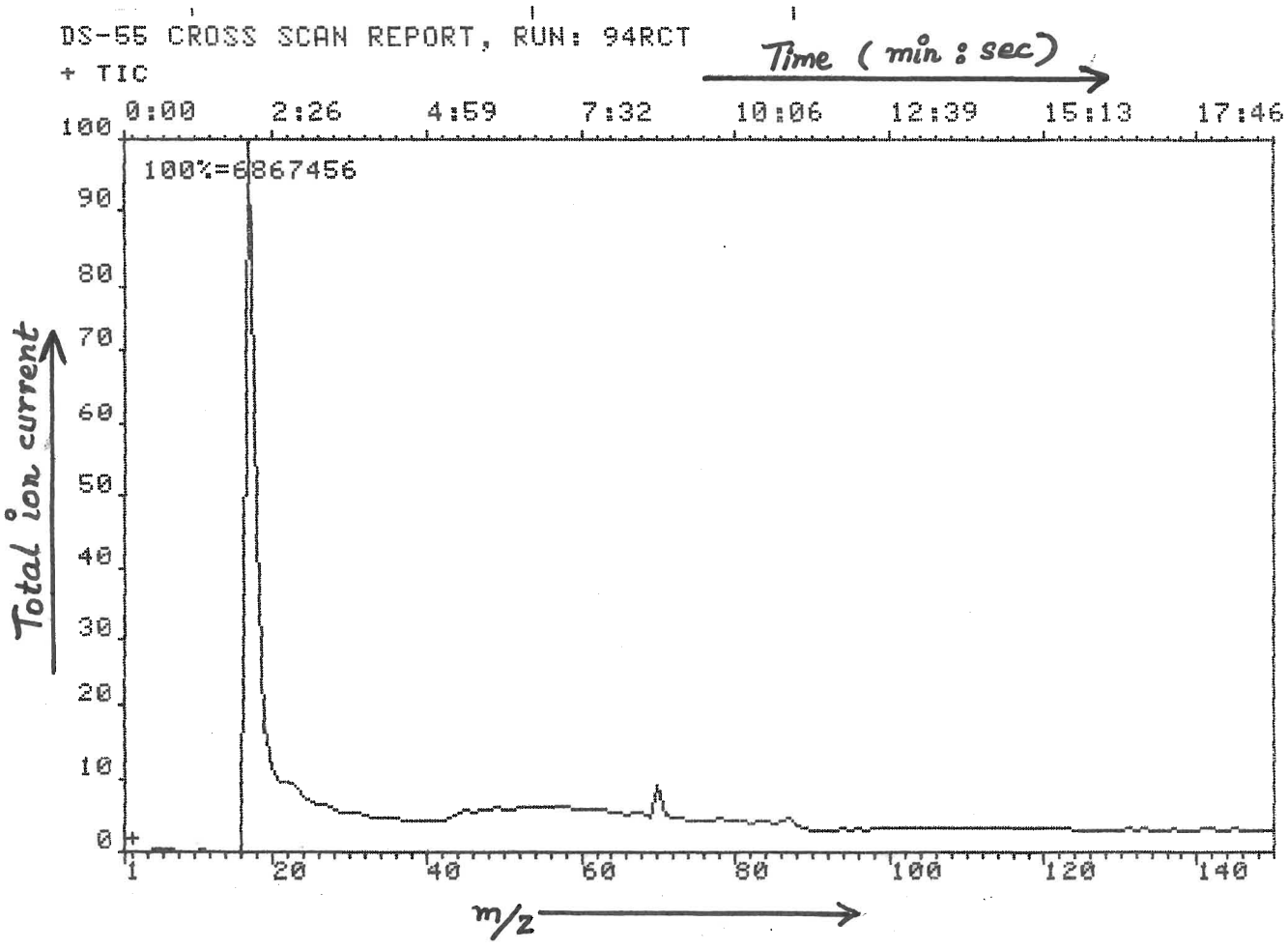


Figure 28(A) Gas chromatogram of methyl ester of 2-(4-chlorophenyl)-isovaleric acid.

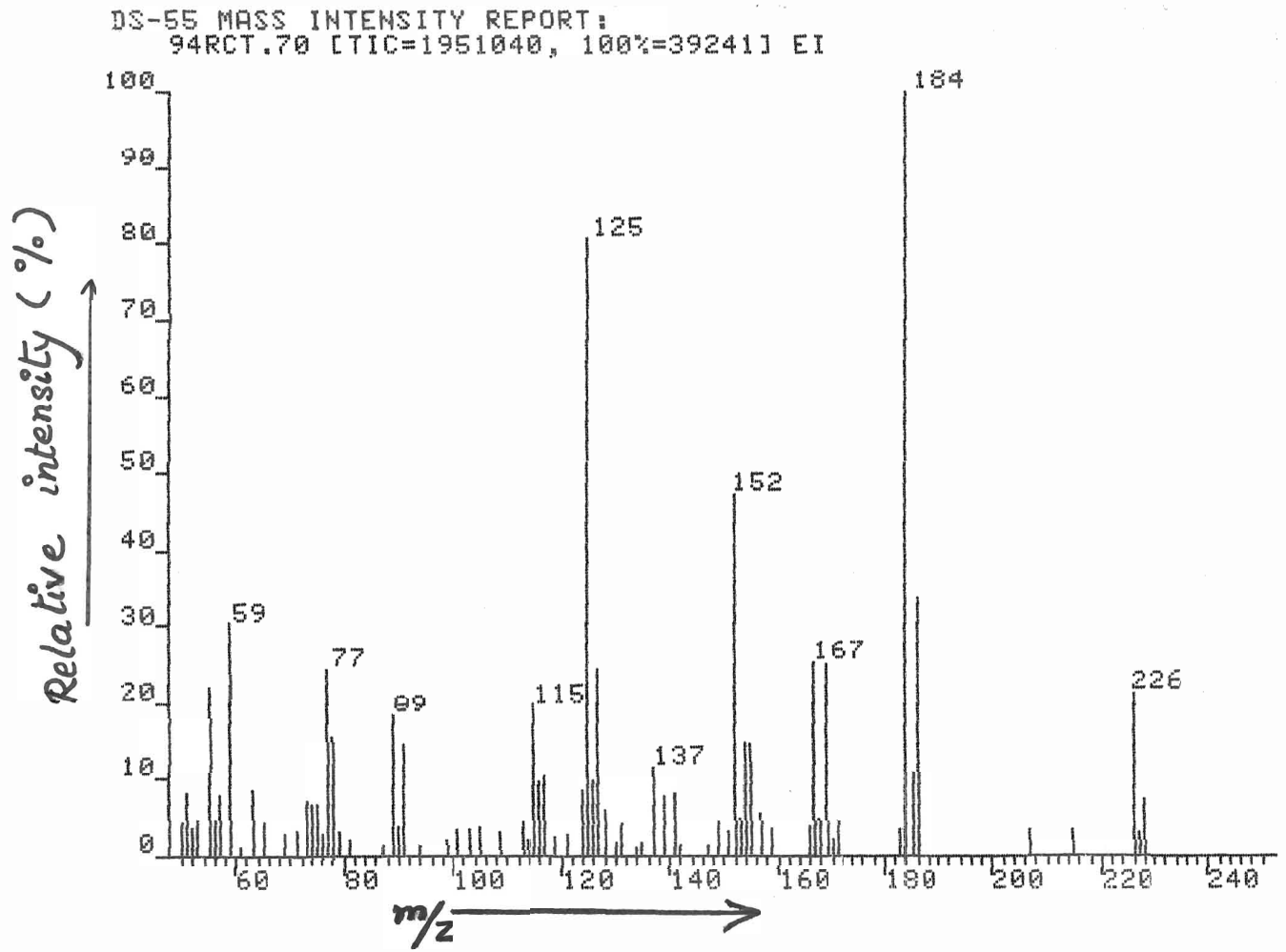


Figure 28(B) Mass spectrum of methyl ester of 2-(4-chlorophenyl)-isovaleric acid.

2. Quantitative determination of phenacyl esters of acid constituent of permethrin by high performance liquid chromatography

As the minimum detectable levels of methyl esters of acid constituent of permethrin was relatively high in the GC-ECD studies, an attempt was made to derivatize the analytical standard acid (Cl_2CA (18), cis:trans ≅ 40:60) with α-bromoacetophenone, using dimethylformamide as the solvent and in the presence of potassium fluoride. A similar reaction was carried out in the absence of the acid component, in order to detect the background of the reagents. Since the phenacyl esters contain the chromophore, the derivative products could effectively be analysed by HPLC with a UV detector.

For the studies, the HPLC with a Milton Roy pump was used and the derivative products were analysed on a Brownlee Labs RP-18 column with a mobile phase of 60% acetonitrile/40% water. Under these conditions, 1.9 μg/mL of the phenacyl ester of cis-Cl_2CA (18) showed a peak at 12.8 min with a 77.5 mm peak height whereas 2.9 μg/mL of trans-Cl_2CA (18) revealed a peak at 11.4 min with a 116 mm peak height. With the average noise level of 1 mm, the minimum detectable amounts of the cis- and trans-esters were calculated as 4.9 ng and 5.0 ng, respectively.

3. Quantitative determination of trichloroethyl esters of acid constituents of permethrin and fenvalerate by gas chromatography with electron capture detection

Although the standard acids, Cl_2CA (18) and CPIA (31), were derivatized to more sensitive methyl esters to improve the detectability

by GC-ECD, the sensitivity of these derivatives was found to be still poor for detection of trace amounts of those residues expected in treated leaf samples. Hence, another attempt was made to derivatize these standard acids with a mixture of trichloroethanol and trifluoroacetic anhydride. Since the esters thus produced have an additional three chlorine atoms, it was expected that these derivatives could increase the sensitivity to much lower minimum detectable levels than those of the methyl esters. In the reaction, however, trifluoroacetyl derivatives could also form, but the formation of these derivatives was insignificant[75] and they were relatively insensitive towards GC-ECD.[84] A similar reaction was also carried out in the absence of the acid component in order to detect the background of the reagents. During the studies by GC-ECD on a 3% OV-275 column at 130°C, 1 µL of 0.009 µg/mL cis-trichloroethyl ester revealed a peak at 1.7 min with a 12.7 mm peak height, whereas 1 µL of 0.013 µg/mL trans-ester showed a peak at 2.2 min with a 17.3 mm peak height. With the average noise level of 1.0 mm, the minimum detectable amounts of cis- and trans-trichloroethyl esters were found to be 0.001 ng and 0.002 ng, respectively.

A similar derivatization was carried out with CPIA (31) also and the product was analysed at a column temperature of 140°C. From the study, 1 µL of 0.07 µg/mL of the trichloroethyl ester of CPIA (31) revealed a peak at 1.6 mm with a 46.5 mm peak height, from which the minimum detectable amount was calculated as 0.003 ng.

VII. Quantitative determination of expected conjugated alcohols and acids of permethrin and fenvalerate

The ether extracts of samples treated with permethrin and fenvalerate (fraction No. 3, Experimental section IV.3), in which conjugated alcohols (PBalc (19) and PBald (38)) and conjugated acids (Cl_2CA (18) and CPIA (31)) were expected to be present, after the cleavage of conjugated materials from acid and alcohol constituents of parent compounds, were cleaned up by two-dimensional preparative TLC plates prior to analysis by GC-FID or MS. The standard and untreated samples were also studied in the same manner as above. All the procedures including the cleavage of conjugated compounds were described previously in Experimental sections IV.3 and IV.4.b.

1. Quantitative determination of expected conjugated alcohols of permethrin and fenvalerate by gas chromatography with flame ionization detection

After the TLC development of ether extracts of 23 day and 84 day permethrin treated samples, the area where PBalc (19) was expected to be present on the preparative TLC plate was extracted with carbon disulphide and the extract was analysed by GC-FID. The sample solution was prepared at the time of injection so that 1 mL of carbon disulphide contained the extract which was equivalent to 1 g of leaf.

From the study, as mentioned in Section II.3, 1 µL of 10.6 µg/mL PBalc (19) showed a peak at a retention time of 3.3 min with a peak height of 40 mm on a 3% OV-275 column at 180°C; from the result, the minimum

detectable limit of this compound was calculated as 0.6 ng. However, under the same conditions, the control and treated leaf samples did not show any peak in the region of interest. It was concluded, therefore, that there was no PBalc (19) in treated leaf samples, or its concentration, if there was any, was lower than the detectable limit of 0.6 µg/g in the leaf.

In the same manner as above, a standard PBald (38) solution with an injection of 1 µL of 6.1 µg/mL gave a peak at 1.7 min with a 29 mm peak height at a column temperature of 150°C. From the results, the minimum detectable amount was calculated as 0.4 ng. Under identical conditions, the control and treated samples did not show any sign of a peak in the region of interest, it was concluded that there was no PBald (38) in the treated leaf samples or its concentration, if present, was lower than 0.4 µg/g in the leaf.

2. Determination of expected conjugated acids of permethrin and fenvalerate by mass spectrometry

After the TLC cleanup of ether extracts of permethrin treated leaf samples, the area where the acid, Cl_2CA (18), was expected to be present, was extracted with methanol and followed by evaporating the solvent to dryness under a gentle air stream. Then the residue thus produced was analysed by mass spectrometry using direct inserting inlet system. The mass spectra of treated samples did not show any fragment peaks related to that of standard acid, Cl_2CA (18); instead the spectra were very similar to that of the control sample.

The fenvalerate treated samples were also studied in the same manner as above, but the mass spectra of these samples did not reveal any peaks related to CPIA (31).

The above observations indicated that the expected acid degradation products, Cl_2CA (18) and CPIA (31), were not present in the treated leaf extracts or their concentrations were too low to detect by this method, even if they were present.

VIII. Quantitative determination of expected esters of free and conjugated acids of permethrin and fenvalerate

The ether extracts of 84 day permethrin treated samples (fractions No. 2 and No. 3, Experimental section IV.3) in which Cl_2CA (18) (present as a free acid and a conjugated acid, respectively) was expected to be present, were treated for esterification with a mixture of trichloroethanol and trifluoroacetic anhydride. The detailed reaction procedures for this derivatization were already mentioned in Experimental section V.3.

The same fractions of 84 day fenvalerate treated samples were also derivatized in the same manner as above. All of the trichloroethyl derivatives thus expected to be produced were analysed by GC-ECD.

Other esterification reactions were carried out with α-bromoacetophenone; the ether extracts of 84 day permethrin treated sample, as mentioned above, were derivatized with α-bromoacetophenone and the products were analysed by HPLC. The detailed reaction procedures were already discussed in Experimental section V.2.

1. Quantitative determination of expected trichloroethyl esters of free and conjugated acids of permethrin and fenvalerate by gas chromatography with electron capture detection

After the ether extracts of 84 day permethrin treated samples were esterified, the products thus produced were analysed on a 3% OV-275 column at the temperature of 130°C by GC-ECD for detection of expected trichloroethyl esters. Under the studied conditions, the ether extracts of both fractions (fractions No. 2 and No. 3 in Experimental section IV.3) did not show any signals at the retention times of trichloroethyl esters of standard cis- and trans-Cl_2CA (18) at 1.7 min and 2.2 min, respectively.

Similarly, the ether extracts of 84 day fenvalerate treated samples were esterified and analysed at a column temperature of 140°C for detection of expected trichloroethyl esters of CPIA (31), but no signal was observed at the retention time of the standard trichloroethyl ester of CPIA (31) at 1.6 min.

The above observations indicated that the expected acid, Cl_2CA (18), was not present in the form of free acid nor in the form of conjugated compound in 84 day permethrin treated sample, or, if present, the concentrations of cis- and trans-trichloroethyl esters were extremely low, being less than the minimum detectable limits of 0.001 ng and 0.002 ng, respectively. The same conclusion was made for 84 day fenvalerate treated sample; the acid, CPIA (31), was not present in the form of free acid nor in the form of conjugated compound, or the amount was less than the minimum detectable limit of 0.003 ng, even if it was present.

2. Quantitative determination of expected phenacyl esters of free and conjugated acids of permethrin by high performance liquid chromatography

Fractions No. 2 and No. 3 (Experimental section IV.3) of the 84 day permethrin treated sample after esterification, in which phenacyl esters of Cl_2CA (18) were expected to be present, were analysed on a Brownlee Labs RP-18 column by using a mixed mobile phase consisting of a 60% acetonitrile and 40% water.

Fraction No. 2 of the above did not show any peaks at the retention times of the phenacyl esters of standard Cl_2CA (18) (cis:trans ≅ 40:60), 12.8 min and 11.4 min, respectively. However, the other reaction product (fraction No. 3) showed the peaks at the regions of interest with peak heights of 2.0 mm and 6.5 mm for the cis- and trans-esters, respectively. These results indicated that the concentrations of acids present as cis- and trans-conjugated acid compounds in the 84 day permethrin treated sample were found to be 0.25 µg/g of leaf and 0.8 µg/g of leaf, respectively.

Summary and conclusion

Since previous studies were conducted only with the radioactive compounds under artificial experimental conditions, this study was conducted with non-radioactive permethrin and fenvalerate in the Niagara region of Ontario. The main objectives of this study were to investigate their persistence and identify their major degradation compounds in apple foliage after spray application of these compounds in an apple orchard. These newest synthetic pyrethroids, permethrin and fenvalerate, were registered in 1978 and 1980, respectively, on a temporary basis for limited use in Canada and their use has been increasing consistently. There have been some concerns, however, about the use of these pesticides, because their persistence was suspected to be much longer than reported, and there would be a possibility of destroying the balance of the natural ecology. It was, therefore, necessary to investigate the real persistence of these compounds including the quantitative determination of degradation compounds, if they were present.

The analysis of residues of cis- and trans-permethrin in apple foliage was done by GC-ECD without any cleanup; as no interference was observed from the leaf co-extractives. The initial deposits of cis- and trans-permethrin were 13.5 ppm (μg/g of leaf) and 19.2 ppm, respectively. Residues declined to 4.0 ppm and 7.9 ppm 23 days after spray application; these values are 29.6% and 41.1% of the initial deposits for cis and trans isomers, respectively. Corresponding values for the sample of 84 days after spray application (at harvest) were 2.7 ppm and 4.7 ppm,

which are 20.0% and 24.5% of the initial deposits, respectively. From these results, the observed half-lives of cis- and trans-permethrin were found to be 42 days and 46 days, respectively.

Since fenvalerate showed a single peak on a 3% OV-275 column used, its quantitative determination was done by the peak height measurement of this single peak. The initial deposit of fenvalerate in apple foliage was 28.0 ppm and the residues declined to 13.4 ppm and 8.0 ppm for the samples of 23 days and 84 days after spray application, respectively; these values are 47.9% and 28.6% of the initial deposit, respectively. From these results, the observed half-life of fenvalerate was calculated to be 51 days. These results indicate that under the same experimental conditions, fenvalerate was slightly more persistent than permethrin.

Several studies[54,57,66] of the metabolic fate of ^{14}C-labelled permethrin and fenvalerate reported that the ester cleavage of these insecticides was the predominant reaction, and as a result of this cleavage, PBalc (19), Cl_2CA (18), PBald (38) and CPIA (31) were produced as the major degradation compounds. However, the majority of these products were then converted to conjugated compounds with plant constituents such as glucose.

The minimum detectable amounts of PBalc (19) under the experimental conditions used were 7.3 ng and 0.6 ng in HPLC and GC-FID, respectively. However, detection of PBalc (19) in the treated leaf samples was impossible because of the strong interference from the leaf co-extractives. As a result of cleanup by two-dimensional TLC, the above interference was eliminated, but no PBalc (19) was detected. Hence the conclusion was

that in the actual leaf samples, the alcohol, PBalc (19), was not present in the form of free alcohol nor in the form of conjugate, or its concentration was lower than the detectable limit of 0.6 ng, if it was present.

Similar conclusions were made in connection with the alcohol, which was reported to be converted and detected as PBald (38), as shown by results of analysis by GC-FID. The lowest detection limit of PBald (38) was 0.4 ng by the GC-FID study.

The standard acids, Cl_2CA (18) and CPIA (31) were derivatized to methyl esters, phenacyl esters and trichloroethyl esters prior to analysis by GC-ECD and HPLC. Since the sensitivities of methyl esters of cis- and trans-Cl_2CA (18) were extremely poor by GC-ECD, being 27.0 µg/g and 23.9 µg/g of leaves respectively, no further attempt was made to pursue this derivatization with the treated samples; there was no possibility of finding this level of residues in the treated samples.

The minimum detectable amounts of the phenacyl esters of the standard acid, (Cl_2CA (18), cis:trans ≅ 40:60) were 4.9 ng and 5.0 ng, respectively. With this level of sensitivity, 0.05 µg/g of leaf of these acids could be detected by HPLC in the treated leaf samples. There was no sign of residues of these acids in the form of free acids, but 0.25 µg/g and 0.8 µg/g of leaves of cis- and trans-Cl_2CA (18) were found in the form of conjugated acids in the 84 day permethrin treated sample.

The minimum detectable amounts of trichloroethyl esters of the standard acids, Cl_2CA (18) (cis:trans ≅ 40:60) and CPIA (31), by GC-ECD are 0.001 ng, 0.002 ng and 0.003 ng, respectively. Two fractions of the

permethrin treated sample (84 days after spray application), in which the above esters were expected to be present, did not show any sign of the presence of esters. It is concluded that there was not any Cl_2CA (18) present in the form of free acid nor in the form of conjugated acid, or, if present, these concentrations were extremely low, being less than the minimum detectable limits of 0.001 ng and 0.002 ng for cis- and trans-trichloroethyl esters, respectively.

From the HPLC results, Cl_2CA (18) was found in the form of conjugated acid, it was thus expected that the trichloroethyl ester of Cl_2CA (18) would also be found because the sensitivity for the trichloroethyl ester by GC-ECD was much better than that of the phenacyl ester by HPLC. The following are some of the possibilities for causing this discrepancy.

Firstly, when the esterification reaction was completed, the reaction product was extracted with hexane prior to analysis quantitatively by GC-ECD. The extracted ester might have been lost, at least to a certain extent, during this extraction.

Secondly, throughout this study, the esters formed from the standard acid were not isolated, all the calculations were based on the weight of starting standard acids with the assumption that the conversion to ester was quantitative, though this was rather difficult to attain. Depending on the efficiency of the reaction, the concentration of ester, based on the yield of product formed, would be quite different.

Thirdly, the standard esters were prepared at high concentration, about 4000 ppm, but the concentrations of expected acids in treated samples were substantially lower, less than 1.0 ppm. Thus, at lower

concentrations, the yield of expected esters would be much lower; especially with the presence of a large quantity of leaf co-extractives when the esterification reaction was performed.

Similar results were obtained with 84 day fenvalerate treated sample; the trichloroethyl ester of the expected acid was not observed. It was concluded, therefore, that there was no CPIA (31) present in the form of free acid, nor in the form of conjugated acid, or if present, its concentration was less than the minimum detectable limit of 0.003 ng.

The results of this study provided firsthand information regarding the persistence and fate of permethrin and fenvalerate under Canadian climatic conditions. Although the values of half-life were shown as 42-51 days, these values were obtained on the basis of total loss of parent compounds due to different factors such as evaporation,[85] wash-off by rain,[86] dilution as a result of leaf expansion,[58] and photochemical degradation. Since the loss due to the first three factors are fairly substantial and the concentrations of expected degradation compounds in this study were extremely low or not detectable, it is concluded that the degradation of permethrin and fenvalerate in apple foliage is very slow. The obtained information may be used for making recommendations as to the use of these chemicals with various crops under different environmental conditions.

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Appendix

Using an advanced capillary GC-MS system (Hewlett Packard GC model HP5880A and Mass Selectivity Detector model HP 5970), further analysis was carried out with the trichloroethyl ester of CPIA (31). The following column and conditions were used in the studies. A fused silica capillary column coated with OV-1 (cross-linked), 0.2 mm I.D., 25 m, operating temperature was programmed from 80°C to 180°C, at a rate of 10°C/min. The amounts of samples injected were 660 ng.

In the studies, fenvalerate treated samples (84 days after spray application) showed molecular ions which correspond reasonably well with that of the standard trichloroethyl ester of CPIA (31).

Samples	Mass	Abundance (%)
1. Trichloroethyl ester of CPIA (31)	341.95	7.9
	343.95	9.9
	345.85	5.3
	347.85	1.1
2. 84 day fenvalerate treated sample (from conjugated acid)	341.85	5.1
	343.85	8.9
3. 84 day fenvalerate treated sample (from free acid)	341.95	7.6
	343.85	10.5
	345.85	5.1

As the system is not accurate enough to record lower values (abundance of less than 5%), the abundance values are not identical, but major values are close enough to prove the presence of expected compounds of free and conjugated acids, CPIA (31), of trichloroethyl ester in the both samples. Since no quantitative work was done, it is impossible to quantify, but the concentrations of these acids in the sample were very low.